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Plant sap analysis - a literature study

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PREFACE

This literature study is made because I wanted to start research on plant sap analysis. The subject is quite complicated and I wanted to have an insight into the work which has been done. I thank M. Prasad for correcting the English.

1. INTRODUCTION

Plant sap analysis has been used for several purposes and in a number of countries: For example

- The Netherlands, for nitrogen top dressing of potato.
- Germany, for nitrogen top dressing of winterwheat and barley.
- Sweden, for fertilization recipe of various crops (Mansson).
- United Kingdom for nitrogen fertilization of vegetables in greenhouses and in the field.
- Spain for vegetables in greenhouses and in the field.
- Guernsey for fertilization recipes of vegetables and flowers under glass.
- France for fertilization of tomato.

The advantages and potentials of plant sap analysis are as followes.

1. Vielemeyer et al. (1991) used plant sap analysis to get an insight into the NO_3 content of lettuce in relation to human health.
2. A number of workers used it for advice on the nutrition of crops. Morard et al. (1991) have been used plant sap analyses for 10 years for tomato and cucumber. Petioles or side shoots were sampled every 10-15 days and analysed by the ENSAT (= Ecole Nationale Supérieure Agronomique de Toulouse, France). Concentration of macro elements were being compared with two earlier sampling dates. The initial work in France was done by Routchenko (1967). He sampled leaves of maize during the 3 to 4 hours after sunrise. If neccessary he cleaned the leaves with alcohol using a towel, put the samples in a bottle with 50 ml ether at a temperature of -30 to -35 ^{o}C. This was the beginning of plant sap analysis in France. Side shoots were sampled, because growers did not accept taking petioles or leaves. Coltman (1988) made the following advice for tomato. Samples of 15 plants have to be taken every week. If NO_3-N in two connected sampling dates is lower than 800 mg/l, the N-concentration in the nutrient solution has to be increased by 50%. Further, the NO_3-N content has to be lower than 1200 mg/l. Otherwise, it is excess. Smith (1987 and 1988) used plant sap analysis for several crops. He was able to see an induced K-deficiency in tomato after a sudden decrease of the K-supply. Long (1982) in the UK had graphs for sprout, cabbage, leek, lettuce, carrott, spinach and onion for optimal NO_3 levels in petioles of fully expended leaves in relation to size and development of the plants. Vermeulen (1988) proposed a quick sap test for blanching celery. Wollring and Wehrmann (1981) used NO_3 content of the basal stem of wheat to give advice for N-supply. Vielemeyer and Weissert (1990a) used weekly plant sap analysis of tomato and cucumber together with analysis of leachate of substrates to adjust the composition of the nutrient solutions. Drews and Fischer (1992) saw plant sap analysis as a complementary to substrate analysis, not as a substitution. Huett and Rose (1988) found the NO_3 content of the youngest fully opened leaf of tomato a sensitive indicator of N status better than total N concentration of leaf plus petiole. Brumagen and Hiatt (1966, cited in Marschner, 1986), found only the soluble Ca fraction an appropriate method to assess the calcium nutritional status of the

buds of Burley Tobacco.

3. Plant sap analysis is a rapid method. If potato petioles are delivered before 10.00 a.m. an advice for N fertilization can be available in the afternoon (Anonymus, 1992).
4. Several authors mentioned the problems of expressing nutrients contents on dry matter basis of the plants, because dry matter contents can change. For elements only present in plant sap this is not accurate. K content expressed per unit of sap was independant of N-, P- and Na-fertilization level and moisture content of the soil (Leigh and Johnston, 1983b). But K content on a dry matter basis was dependent of these factors, because they had an influence on the dry matter content. In four experiments it was found that K content of 6-9% on dry matter basis decreased to 2% during 130 to 180 days after sowing. The K content in the sap stayed stable at 200 mM. Only in two experiments the last 20 days before harvest the K content in the sap increased to 500 mM. Sonneveld and De Bes (1983 and 1988) found in the winter lower dry matter contents than in summer, leading to higher K contents per unit of dry matter in the winter than in the summer. Expressing K contents per unit of sap showed stable contents during the whole year. Scaife and Bray (1977) concluded that it is better to analyse sap rather than dry matter plant tissue and expressing it as nutrient concentrations in the sap. The dry matter method is more subject to 'noise', since there exist differences in dry matter content between growing methods and species. Leigh and Johnson (1983b) assumed that phosphorus concentrations in tissue water is more useful than in dry matter for assessing the P status of crops and may have some advantages over content in dry matter because they are less sensitive to factors such as N fertilization. Cassidy (1966 and 1970) showed that K and Cl should be reported on a rational basis such as normality and not as a % in the dry matter content. The dry matter content is sometimes quite different from plant part to plant part, or during the growing period. Leigh et al. (1982) concluded that for barley the tissue water provides a better basis than dry matter for calculating tissue nutrient concentrations for P and K. For N it was not clear which method is the best. K contents on tissue water basis is also used to determine optimal K contents of several crops by De Kreij et al. (1992).
5. Plant sap analysis can be used as a diagnostic tool. Routchenko (1971) recognized Zn deficiency in maize induced by high P. Bar-Akiva (1984) found total-Fe content was not suitable to assess Fe status of plants. Peroxyde-enzyme activity was a good indication of Fe status. Rahimi and Schropp (1984) found Zn in plant sap a better indicator of Zn status of the plant than total Zn content in plant material.
6. Burns (1992a) omitted at a certain stage either N, P or K in fully fertilized plants and found that plant sap concentration reacted very quickly to this change of fertilization. So plant sap analysis is a sensitive method. Peck et al. (1974; cited in Scaife and Bray, 1977) illustrated the advantage of measuring sap NO_3 relative to total N in table beet (Table 1). Sap NO_3 was more sensitive than total N (dry matter).

Table 1. Ratio of sap NO_3 or total N in high N plants to zero-N plants (Peck et al. 1974).

	Ratio	
	sap NO_3	total N
leaf blades	9	1
petioles	16	2

There were not only positive results with plant sap analysis but also negative experiences. A disadvantage was the high coefficient of variation (Burns and Hutsby, 1986b). Lettuce was sampled and analysed in triplicate. Coefficients of variation were as follows: total-K content on dry matter 6%, K-sap in young petioles 6-12%, K-sap in intermediate age of petioles 14%, K-sap in petioles of old leaves 19-29%.

Vielemeyer and Weissert (1990c) tried to predict blossom-end rot of tomato from Ca in plant sap, but they were not succesful. The reasoning was that only Ca in water soluble plant sap was available for fruits. Therefore they tested if Ca in the sap of expressed petioles was related to the amount of blossom-end rot. They could not find any relationship for sap Ca nor for total Ca. Blossom-end rot in the six treatments was 1.2 - 10.6 % of the total harvest and Ca in petioles was between 150 and 670 mg/l in young leaves, 260-640 mg/l in middle leaves and 410-930 in old leaves.

Haarstrich (1988) could not distinguish N excess in cyclamen. Plants showed negative effects of N excess (shoot growth in relation to flowers) and this affected the quality. NO_3 content in petioles of plant with excess was not different from NO_3 in plants with optimal N supply.

Schulz and Marschner (1987) stated that the nitrate-quick test did not work when N was taken up as NH_4. In that case amino-N-test was a better indicator.

Prasad and Spiers (1982) compared growth rate and dry weight of pot plants on different nitrogen levels with NO_3 contents in petioles. The correlation coefficients were not high: R_2 between 0.4 and 0.8. For total-N better correlations were found: R between 0.6 and 0.9.

Verwer et al. (1990) could not find a good relationship between optimal N fertilization and NO_3 in plant sap. However the N-min method in soil was not better either.

If the soil is dry then low NO_3 contents in plant sap was found (Scaife, 1979b). Scaife and Turner (1987) conducted 46 trials with commercial vegetable crops, twenty of them with brussels sprout. The aim was to find a relationship between sap NO_3 and optimal N top dressing. The sap test was incapable of predicting the optimal top dressing.

Scaife and Barnes (1977) reconstructed the results of Ulrich (Fig. 1).

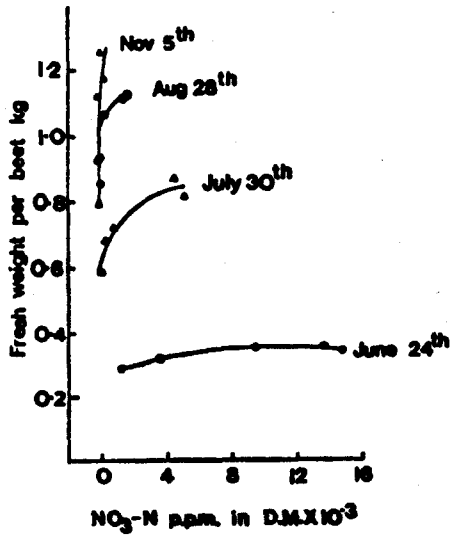


Fig. 1. Relationship between NO_3 content in sap from different sampling dates and final sugar beet yield

Ulrich grew sugar beet at five levels of N and sampled for beet yield and nitrate status of mature petioles at four growth stages. In the young crop there was a very wide range of petiole nitrate levels but little variation in yield. At intermediate growth stages a very clear positive correlation existed between yield and petiole nitrate, the critical level of the latter being high, and as crop maturity approached there was a wide range of yield values but all petiole nitrate levels were low and scarcely related to yield. So, it is difficult to define the critical NO_3 level in petioles.

Certain species such as members of the Rosaceae, reduce most of their N to amino-acids and amides in the roots so very little nitrate is to be found in shoots (Scaife and Stevens, 1977). Schenk (1988) came to the same conclusion: plant sap nitrate is only useful to characterize N-status of plants which do not reduce nitrate mainly in roots. This is the case for example for begonia. Cyclamen reduced three fold higher amount of N in the roots than in the leaves, therefore Cyclamen NO_3 in plant sap of leaves was a good indicator for the N-status. Saintpaulia showed an intermediate place: nitrate reductase activity in leaves was equal to that in roots.

Scaife et al. (1985, Annual Report, National Vegetable Research Station, Wellesbourne, U.K.) conducted 19 field trials on Brussels sprouts, cabbage greens, onion, leeks, cabbage and spinach with N top-dressings. The prediction of N top-dressing by the sap test was disappointing in relation to the experimentally found N requirements.

Scheunemann and Pashold (1989) could not find a good relationship between optimal N-fertilization and sap analysis for white cabbage, late carrot, gherkin and autumn leek.

Poehlman (1935) related NO_3, P and K contents of expressed whole soybean plants to the production. There was no correlation between these factors. Probably the large variation due to seasonal factors makes the establishment of critical concentrations very problematical.

2. METHODS

2.1. Sampling

The method of sampling influences the results. So standardization of sampling is important. The factors involved in the standardization are time, leaf parts and amount of plant material.

Alt and Füll (1988) took lettuce samples 4.30 hours after sunrise. Coltmann (1988) sampled at 11.00 a.m. or for the K content of tomato between 8 and 9 a.m. Morard and Kerhoas (1987), who used the technique according to Routchenko, sampled the three hours after sunrise. Kroon (1990) and Willemse (1991) sampled petioles of potato before 10.00 a.m. in a dry crop. Scaife (1979a) sampled between 10.00 a.m. and 4.00 p.m. after some sunny days but later on he stated that daily variation was small, so sampling time is not so important (Scaife et al., 1983).

Considering the plant parts, some authors prefer laminae, other petioles, stems or side shoots. Plant parts sampled can be of old, young or of intermediate age. Routchenko (1967) sampled the entire stem of tomato during the first flowering, or the petiole opposite the first truss, one month after flowering. For cucumber the stem and petioles are sampled during planting time or during the 9-leaf stage, the petiole of the 5^{th} leaf or during 22-leaf stage, the petiole of the 8^{th} leaf. This method has also been used by Morard and Kerhoas (1987).

Wollring and Köhler (1989) sampled 0.5 cm of the petiole or middle lamelle of old leaves of at least 20 plants. Lucas and Wittwer (1963) sampled petioles of leaves under the last truss for tomato. Drews and Fischer (1992 and 1989) took petioles of the 3^{rd} -5^{th} leaf from the top of tomato. They did not take the youngest leaves because element contents in the young leaves are influenced too much by nutrient excess or by deficiency. Concentrations in the more older leaves are more stable. For cucumber they sampled 5^{th} leaf from the top and later on side shoots. One petiole per plant from at least 20 plants has to be sampled. Scaife (1979b) sampled petioles. Lyons et al. (1991) took petioles of youngest fully grown mature leaves.

Coltman (1987, 1988) sampled tomato petioles of young fully grown leaves for K and NO_3 content. The variation in NO_3 content from plant to plant was great. Therefore at least 10 to 20 plants have to be sampled. For K the variation from plant to plant is less than for NO_3, so the amount of sampled plants can be less. Prasad and Spiers (1985) took petioles of youngest fully grown leaves of tomato and pot plants (Prasad and Spiers, 1982).

Scaife and Bray (1977) recommended the petiole of the youngest fully expanded leaves. But the degree of leaf expansion is less important once one avoids inclusion of proteinaceous material (leaf blades) in the analyses. Therefore they recommended petioles. Scaife and Stevens (1983) found large differences in NO_3 concentrations between cabbage plants, especially for the lower leaves. Therefore they recommend to sample not lower leaves but 'recently matured' leaves, and a minimum number should be 20 plants.

The technique of Månsson is to sample old leaf parts. These old leaf parts work as a buffer for N, P and S of young leaves. Young leaf parts always get enough nutrients, because they can be fed by the base leaves. Comparing 'base' leaves with 'top' leaves gives better information. Mol (1993) and Stijger (1993) mentioned the sampling technique of Månsson:

15 to 20 leaves of matured leaves hanging in the sun, halfway from the base and the top of the plant. For tomato and cucumber petioles and thick middle nervature and for sweet pepper petioles plus laminae are taken. For tomato normally the 'base' leave is sampled (about 2 m from the top) if sampling is frequent. For occasional sampling it is better to take also the 'mid' leaf (1 m from the top). NO_3 in the 'base' leaf has to be higher than in the top leave. This technique is also used for cucumber. For roses Månsson (1991, personel communication) recommend to sample the first lowest five finger leave (counted from the base of the stem) of stems with a young flowering bud (Figure 2), except when the stems are long. Then the 6th leave with five fingers from the bud is sampled and 50-80 leaves are taken.

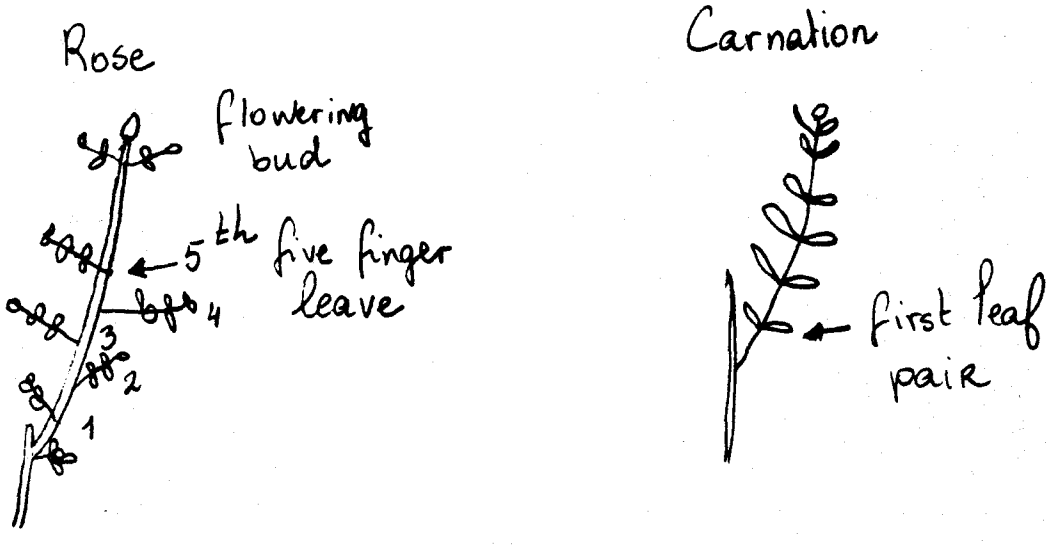


Fig. 2. Recommended leaf for sampling of rose and carnation (Månsson, 1991, personel communication)

For carnation the first leaf pair of a stem with a flower at the start of the budding stage has to be taken (Fig. 2) and 80-100 leaf pairs are taken. For tomato 12-16 leaf petioles are taken, for cucumber 6-8 petioles with the laminae removed. Petioles are cut in pieces of about 10 cm packed in air free plastic sacks. For chrysanthemum old active leaves are taken, weighing at least 75 g. For lettuce the whole plant is sampled. The method of Månsson is subscribed by Vielemeyer and Weissert (1990) who have the opinion that young side shoots, as sampled by Morard and Kerhoas (1983) are not suitable, because they always get enough nutrients. Morard and Kerhoas (1983 and 1987) sampled these side shoots because then the plants are not damaged and is therefore acceptable to the growers. This sampling technique is used for tomato and cucumber. 10 to 20 plants and one side shoot per plant with a length of 10-15 cm and a maximum diameter of a pencil are taken. To avoid boundry effects the sides of the field or greenhouse are not sampled. They compared nutrient contents of these side shoots with nutrient contents with pieces of the

stem on the same height where they took the side shoots. There were no differences, so the method to sample only side shoots was convenient. Burns and Hutsby (1984 and 1986b) used petioles cut into pieces of about 3 cm.

Smith (1987) sampled 10-12 petioles of tomato near the ripening truss ('base' leaf) and for the second sample the petiole halfway the first sample and the top of the plant ('mid' sample). Both samples contain 12 petioles. The 'base' sample together with the 'mid' sample is taken if the crop is not sampled regularly. The 'mid' petiole is taken if the crop is sampled regularly. For rose 40-60 leaves of the lowest five-finger leaves are sampled. That is the leaf immediately above the cut of a stem which can be harvested. For cucumber and melon 12 petioles are sampled halfway between the harvested fruit and the growing point. For eggplant and sweet pepper 15-40 petioles are sampled half-way between the harvested fruits and the top of the plant, for carnation 15 young shoots are broken of and of each 5 leaf pairs are taken, and for chrysanthemum 30-40 young fully grown leaves are sampled. For Gerbera 10-20 young, fully grown leaves. For Alstroemeria 30-40 leaves opposite developing flower buds are taken. For freesia it is 40-60 top half leaves of young fully grown leaves.

Vielemeyer et al. (1991) sampled petioles of young fully grown leaves of tomato and cucumber; that is about the 5^{th} leaf from the top. This technique has been followed by Drews and Fischer (1992).

Scheunemann and Paschold (1989) used the following plant parts (counting from young leaves): white cabbage - petioles of 4^{th}, 5^{nd}, 6^{th} leaf; until headforming, counting from inside to outside. After headforming petiole and nervature of three joining leaves closest to the head are taken. Late carrot - petioles of 4^{th} leaf; gherkin - petioles of matured leaves of main stem; autumn leek - matured leaves from 6^{th} leaf. Geyer and Marschner (1990) recommended for maize old leaves to control N fertilization, because old leaves had the highest NO_3 content.

In some cases stems are taken. For example Pettinger (1931) sampled pieces of 15 inch of the stem of maize. Wollring (1983) took 0.5 cm pieces of the lowest stem parts of wheat. Alt and Füll (1988) took from the biggest fully grown leaves the nervature. At least 30 leaves have to be sampled, but 50 leaves are better. The reason why they took the nervature and not the laminae is that the press sap from the nervature is more clear than that of the laminae, and the nervature is easier to press the sap out.

Burns (1988) prefered to sample young leaves because the variability of young leaves is less than of old leaves. To get a coefficient of variation < 5% 80 old leaves have to be sampled. For young leaves less number of leaves can be taken. Adams (1982), Vielemeyer and Weissert (1990), Drews and Fischer (1992) sample at least 5 to 7 leaves. Drews and Fischer (1989) took one leaf at least from 15 plants. Schulz and Marschner (1987) took old and young leaves of winterwheat. Vielemeyer et al.(1991) sampled entire lettuce crop, cut them in quarters, mixed the material, took 30 g which is mixed in a mixer with addition of 270 ml 1% $CuSO_4.5H_2O$ solution. After 3 minutes mixing and filtration NO_3 was measured with NO_3 electrode. Bar-Akiva (1984) took leaf discs of 5-7 mm diameter which be liquified. Martinez-Canadas et al. (1985) sampled 5, 10, 20, 30, and 40 pepper leaves and determined N, P, K, Ca and Mg in the dry matter. To get an error < 15% at least 30 leaves had to be sampled. For N the sampling error was the smallest and for Mg the

biggest. In Spain a general rule is to sample 1.2% of the plants of a certain greenhouse and side rows are not sampled. Morard and Kerhoas (1987) following the method Routchenko (1967) took 10 'organs' (petioles) which is equal to >25 g fresh weight. These are cut into pieces and put in flasks with 50 ml ether at a temperature of -20°C. Samples have to be kept below freezing point. At -15 to -20°C samples can be kept for one or two weeks. Kroon (1990) and Willemse (1991) took petioles every 10 to 14 days, 4 to 5 times per growing season of a potato crop. The first fully developed compound leaf is sampled of 40 plants. Laminae are taken off and petioles are put into plastic bags into the freezer for at least 4 hours. Scaife (1979b) and Scaife et al. (1983) found that the variation between plants and between the age of the organs was quite large. Variation by weather and time of sampling during the day was small. De Bes and Van Dijk (1979) sampled at least 150 g fresh material. They froze the sample, thawed and pressed it. In this way they collect about 100 ml which is enough for analysis. Sonneveld and de Bes (1983) sampled petioles and laminae of tomato, cucumber and eggplant and edges and middle vein of lettuce. Burns (1991 and 1992a) prefered for N, P, K analysis young fast growing leaves. Old leaves act as a buffer (source) for young leaves (sink). So nutrients have to move from old to young leaves when there is a deficiency. This transport is not fast enough to meet the demand of the young leaves. Young leaves react faster to a sudden nutrient deficiency than old leaves. Therefore young leaves have to be sampled. In old leaves there can be a luxurious accumulation. Burns (1988) recommendeded a young mature leaf. However, it can be difficult to recognize what is a young mature leaf. Also, there are for nitrate and potassium higher contents in old than in young leaves. For phosphate the opposite holds. The variation is also different. The coefficient of variation is given in Table 2. Petioles were pressed from 5 pieces.

Table 2. Coefficient of variation of different sap samples (Burns, 1988).

	Coefficient of variation		
Leaves	Nitrate %	Potassium %	Phosphate %
young	19	12	17
middle	15	15	23
old	38	19	27

To get a coefficient of variation <5% 80 petioles have to be sampled. Young leaves are most sensitive to changes in the nutrient supply (nitrate and phosphate) when nutrients are re-supplied after a period of starvation. Young leaves are more sensitive than old leaves in the absence of K and NO_3 when Na and Cl is present. In this situation Na and Cl accumulate in young leaves. The best compromise was to select leaves from the middle part of the plants.

2.2. Pretreatment

2.2.1. Freezing

Freezing is used by many researchers. Routchenko (1967), Morard and Kerhoas (1983 and 1987), Garcia and Galinier (1989) cut the plant materials (petioles or young side shoots of tomato and cucumber) in small pieces and are put in flasks of 50 ml ether at a temperature of -20°C. These flasks have to be transported to the laborotry at a temperature <0°C. In this way they can be kept for 1 week. In the laboratry samples are taken out from the ether and are pressed. In this sap NO_3, NH_4, P, K, Ca and Mg are determined. Lindhauer et al. (1990) froze the sample in liquid nitrogen and kept the sample in deep freeze. After thawing, the sample is milled and pressed, centrifuged at 0°C (17000 rpm) and the sample was kept at -20°C because they analyze also fructose, glucose and sucrose. Lucas and Wittwer (1963) froze the sample at -20°C. When petioles are very hard as for tomato Drews and Fischer (1989) froze it in a plastic bag and after defreezing they pressed it by hand. Kroon (1990) and Willemse (1991) froze petioles of potato for at least 4 hours at -20°C and after defreezing pressed the samples with a fruit press.

Sonneveld and Voogt (1986) froze samples to -30°C and after defreezing they pressed it at 80-100 kPa. The press is made from nylon and PVC. Samples are frozen because more press sap can be collected (De Bes and van Dijk, 1979). In Table 3 the amount of sap produced is given. Freezing increased the K, Mg and P contents of the sap (Table 4).

Table 3. Amount of sap expressed from frozen and unfrozen samples (De Bes and Van Dijk, 1979).

	Amount of sap, relative to fresh weight, %	
	Unfrozen	Frozen
Tomato	15	70
Cucumber	40	75

Table 4. Influence of freezing on contents in the sap (De Bes and Van Dijk, 1979).

	Cucumber		Tomato	
	Unfrozen	Frozen	Unfrozen	Frozen
K, mmol/l	152	185	116	127
Ca	56	49	51	55
Mg	30	42	6	11
NO_3	120	130	142	148
P	6.0	13.1	3.5	7.5

Sap was centrifugated at 8000 rpm for 15 minutes. Sap has to be filtrated and, without acidifying, contents have to be determined within 2 days (Sonneveld and De Bes, 1986). Sonneveld (1980) found that

acidifying the sap with 0.1 M HCl increased the storage ability of the sap, while it had no influence on the contents. Per 50 ml sap 0.2 ml HCl-38% was added. Cl has to be determined before acidifying.

Hunt (1981, Annual Report, National Vegetable Research Station, Wellesbourne, U.K.) used milled and frozen petioles. Sap was centrifugated and 25 ul was diluted by a microprocessor controlled diluter/dispenser.

Vielemeyer and Weissert (1990a) found higher PO_4 and K contents as a result of freezing (Table 5).

Table 5. Influence of freezing of cucumber petioles; relative contents (Vielemeyer and Weissert, 1990a).

	NO_3	PO_4	K	Ca	Mg
Frozen	100	100	100	100	100
Unfrozen	99	66	91	104	88
LSD (p=0.05)	n.s.	5	6	n.s.	n.s.

Månsson froze the samples overnight at -20°C. Freezing had to be done slowly and not for example with liquid nitrogen. Otherwise the positive effect of freezing is not found. The positive effect is that, if freezing goes slowly, ice cristals are being formed which break cell walls. Thus more sap can be easily expressed.

2.2.2. Pressing fresh plant material

Plant material can be pressed directly after sampling. Coltman (1987b) pressed petioles of tomato by a garlic press. The first sap was colourless and the last sap was green and cloudy. There was no difference in NO_3 content of the first and the last sap. He also rolled a pen over petioles to press the sap. Big differences were found between the samples of individual petioles. So, a mixed sample had to be made. Later on, Coltman and Riede (1992) divided petioles of tomato in segments of 1 cm and pressed and diluted 100 ul sap with 900 ml demineralized water.

Drews and Fischer (1989) pressed petioles of tomato and cucumber with a press. Scaife (1979b) sampled petioles of at least 10 plants. Petioles were cut with a sharp knife and put on a hard surface. With a pen sap was pressed on test strips to determine NO_3 content. The colour of the strip after 2 minutes contact with the sap was compared with standards.

Burns (1988) expressed whole plants when they are small using disposable plastic syringes. The saps clarity can be improved by prior freezing the plant material or by passing the sap through a microfilter.

In Spain (Hernando and Cadahía, 1973) pressed fresh material and the sap was deproteinized by adding ethanol.

Adams (1982) crushed plant material using a small hand-press and the sap obtained was cleared by centrifuging.

The Research Station for Horticulture under Glass, Naaldwijk, uses the fresh method for NO_3 analysis in lettuce. Formerly carrez I ($K_4Fe(CN)_6.3H_2O$) and carrez II ($ZnSO_4.7H_2O$) was added to the sample to deproteinize the sap. Nowadays it is analysed after dialysing by auto analyser without adding the carrez.

2.2.3. Mixer-blender

Fresh plant material can be chopped in a mixer-blender. Vielemeyer et al. (1991) took 30 g lettuce with 270 ml 1% $CuSO_4.5H_4O$ solution, mixed it, and after filtration NO_3 was measured with a specific electrode. They also evaluated a second fast method. Middle veins of outer active leaves were pressed with a garlic press. The sap was diluted 100 times and NO_3 was measured with a test strip. The third method involved drying the lettuce and dry matter content was determined. 0.5 g dry material was diluted in 1% $CuSO_4.5H_2O$ solution and shaken. NO_3 was determined and recalculated to fresh weight. The conclusion was that the 'dry matter method' gave lower NO_3 than the fresh 'method'. The relationship was $y = 13.2 + 1.14\ x$; $r = 0.89$, with $y = NO_3$ in mg/kg fresh material by 'fresh method' and $x = NO_3$ in mg/kg fresh material by 'dry method'. If the 'dry method' gave 3000 mg NO_3/kg fresh material than the 'fresh method' gave 3433 mg NO_3/kg fresh. The relationship between NO_3 by the 'dry method' and the press sap of vein of outer active leaves was reasonable ($r = 0.80$).

Smith (personal communication, 1987 and 1988) cut the petioles into 2-3 cm lenghts and a representative 20 g sub-sample was taken. If the crop had been treated with any nutrient sprays the petiole sections were briefly washed in distilled water at this stage but not otherwise. By washing Fe and B were reduced by about 30%, Ca, Mg, Cu, Zn and Mn were reduced by about 5-15%. The level of NO_3-N was increased by about 10%. P, K and Na were not changed by washing. The sample was then transfered to a 1 l domestic liquidiser goblet together with 200 ml distilled water and approximately 0.2 g phosphate-free decolourising charcoal (Darco G-60). The sample was macerated for 20 secs and immediately filtered through a Whatman No. 2 paper. The resulting filtrate was analysed as a nutrient solution. The extract can be refrigerated overnight. For roses leaves were blended using a 100 ml goblet on a Waring blender. The extraction rate was 1:50. Before maceration leaves were cut into 1 cm squares and maceration was done for 60 seconds. Dilution of 1:10 gave a considerably less Na, rather less K, NO_3-N and Ca and more Mg. To clear the extract prior to colorimetric or turbidimetric procedures for example for P and B, 2 ml of 2 M HCl was added to 20 ml extract together with about 0.2 g decoulorizing charcoal. The extract was left for 30 minutes before being filtered through Whatman No. 2 paper. In this extract also Fe, Cu, Zn and Mn was determined. Conductivity of the sap is reported directly. All other analysis are corrected for dilution and for adding the acid-cleared extract. Smith (1988) found that storing samples for 2-3 days gave lower element contents.

The Research Station for Horticulture under Glass uses this method for nitrate analysis. 50 g of for example fresh lettuce is mixed with 200 ml water.

2.2.4. Dry plant material.

Methods in which the plant material is dried is not a part of this study. I make one exception and that is when the dried material is shaken with water to extract nutrients. One example is already mentioned in paragraph 2.2.3.

Reinink and Groenwold (1986) compared a 'dry method' with a 'fresh method'. In the 'dry method' lettuce leaves were dried. Dry matter content was determined. After shaking the dried sample with water, NO_3 was determined. In the 'fresh method' press sap was collected and NO_3 was determined in the sap. Results are given in Table 6. The 'fresh method' gave higher NO_3 contents than 'dry method'.

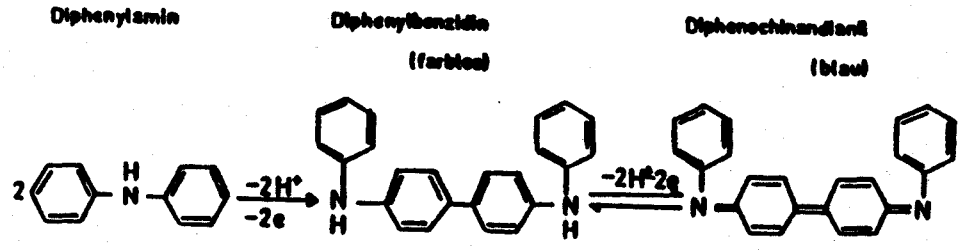
Table 6. Comparison of 'dry method' and 'fresh method' (Reinink and Groenwold, 1986)

Variety of lettuce	NO_3, mg NO_3/kg fresh material	
	dry method	fresh method
Panvit	5000	5200
Pinto	3800	4200
Roland	3100	3600
Romaine verte d'hiver	2900	3300

2.3. Analysis

2.3.1. Nitrate

Nitrate has recieved the most attention. The NO_3 test strip of Merckoquant uses the following principal, which can be executed also with normal chemicals (Wollring, 1983).



Diphenylamine in an acid solution (1 g diphenylamine in 100 ml concentrated H_2SO_4) is oxydized by NO_3 to a blue quinoid imonium ion.NO_3 is reduced to NO_2. The reaction on the strip has to take place for 2 minutes. Then the blue to purple colour has to be compared with standards. If the colour is darker than the maximum concentration 8 mmol/l NO_3 (NO_3-N = 113 mg/l) reaction time has to be measured till it reached the darkest colour. The reaction time, comparing with standards, gives the concentration.

Burns (1988) compared the Merckoquant test strip and the Hach test strip with HPLC measurements. Merckoquant NO_3 contents were 69% of HPLC NO_3 contents (R^2 = 0.76). Hach NO_3 contents were 96% of HPLC nitrate contents (R^2 = 0.92). According to him Hach nitrate test strip is better than the Merckoquant test strip. Scaife (1979) also used the Merckoquant strip.

Alt and Heitkamp (1984) compared NO_3 determination by the

semiquantitative methods - Diphenylamine-H_2SO_4 and Merckoquant test strip - with NO_3 determination by destillation. Midribs of old lettuce leaves were used as samples. Correlation coefficient between Merckoquant and destillation was 0.92 (n=67) and of Diphenylamin-Test and destillation 0.88 (n=67). Merckoquant was better than Diphenylamin-Test. Only at concentrations > 680 mg NO_3-N per 1 Merckoquant gave incorrect values. Merckoquant strips gave no problems with strong coloured sap. In the Diphenylamin-method 1 g diphenylamin is diluted in 100 ml concentrated H_2SO_4. One drop of plant sap is added on a glassplate to four drops of that solution. The blue colour is compared with known KNO_3 solutions. Higher concentration than 450 mg NO_3-N/l have to be diluted.

Wollring (1983) also compared three methods. Lettuce was dried and extracted with water. NO_3 was determinated by destillation, and given per unit of fresh weight. NO_3 was determined in sap by Merckoquant and diphenyl method. Correlation between destillation and test strips was very high (r=0.999 , n=117). Sap gave higher contents than drying. For example by the sap method NO_3 content was 4210 mg/kg fresh and by the dry method NO_3 was 4000 mg/kg fresh.

Vertregt and Rutgers (1984) compared NO_3 determination by Merckoquant and by a Technicon autoanalyzer. 1 part of plant material was mixed with 10 parts water (weight/weight). High correlation between the methods was found between 4.5 and 9.5 mg/l NO_3-N. For leek and onion no colour reaction was found with Merckoquant. With endive, purslane, spinach, cabbage, lettuce, potato, and wheat Merckoquant test strips gave a colouration.

Lyons et al. (1991) pressed leaf petioles of kenaf (Hibiscus cannabis) with a garlic press, diluted it 200 times and determined NO_3 by colorometry. Coltman (1987b) used Merckoquant tests strips for NO_3 determination in press sap of petioles of tomato.

Elliott et al. (1987) took basal stem of wheat in pieces of 1-2 mm. 1 g of the stem was mixed and shaken with 4-6 ml acetic acid and 40 ml vial for 5 minutes. The mixture was kept for two hours and NO_3 was determined by Merckoquant test strip. The strips gave different results depending on the batch. Within one batch the test strips gave the same result. Alt and Füll (1988) solved this problem by verifying each box of test strips with a solution of 100 mg/l NO_3-N. Coltman (1987 and 1988) determined the relationship between the time the test strip of Merckoquant reached the maximum colour and the concentration.

The relationshop was: $\ln NO_3\text{-N} = 10.68 / (\ln t)^{0.513}$.
NO_3-N is in mg/l. A good relationship was found for 80 values (R^2=0.98).

Prasad and Spiers (1984) pressed petioles of young fully grown leaves of carrot, cellery, potato and tomato and stem of maize. NO_3 was determined by Merckoquant test strip. Samples were also dried and extracted with 2% acetic acid and wetting agent (TritonX-100). The most frequent occuring concentrations in the sap were between 200 and 2000 mg/l NO_3-N. Concentrations higher than 114 mg/l NO_3-N were estimated from the time to reach the maximum colour. Relationship and correlation coefficient between the two methods were good (R^2 = 0.8 to 0.9). With the test strip four operators were compared. The coefficient of variation between the operators was acceptably low (c.v. between 10 - 20%).

Drews and Fischer (1989) used the disodium salt of 4,5 dihydroxy-2,7 naphathalindisulfonicacid ($C_{10}H_6Na_2O_8S_2.2H_2O$) in concentrated sulfuric

acid to determine NO_3 content in sap. Sap was filtrated and centrifugated and colour was measured at 436 nm.

Willemse (1991) and Kroon (1992) used the Merckoquant test strip for NO_3. The colour was measured by the reflectometer Nitracheq. Sap has to be diluted 50 or 100 times. Optimal concentrations were 0.81 - 2.42 mmol/l NO_3. Each box of strips had to be calibrated.

Hunt (1981, Annual Report, National Vegetable Research Station, Wellesbourne, U.K.) compared NO_3 by ionselective electrode and continous flow. For young leaves correlation was high, but for old leaves there was no correlation. The reason for the low correlation is not mentioned. He improved the method of the National Vegetable Research Station, Wellesbourne UK, by taking broken petioles and by freezing them. A microprocessor-controlled diluter/dispenser could dilute 25 ul sap. NO_3 was determined by selective ion electrode and a reference electrode, set 0.25 mm apart. In this way a volume of 10 - 100 ul NO_3 could be measured.

Burns (1988) compared NO_3 determination as follows the range 0-35.5 mmol/l NO_3. The correlation coefficients were Hach - HPLC; $R^2 = 0.924$. Merckoquant test strip - HPLC ; $R^2 = 0.756$. Hach colometric determination related better with HPLC than Merckoquant. NH_4 interfered with the determination of NO_3 by Merckoquant. Normally NH_4 in sap is low. Another interference was with low relative molecular mass amines which accumulate in K deficient plants.

Scaife and Stevens (1983) found high correlations between NO_3 in sap measured with Merckoquant test strip and Corning-Eel NO_3 specific ion electrode. To deal with interference Hunt (1985, Annual Report National Vegetable Research Station, Wellesbourne, UK) divised an ion selective NO_3 electrode technique. Interference from organic matter in the sap was partially eliminated by adding 0.05 M aluminiumsulphate solution and activated carbon.

Burns and Griffin (1986/1987) found the Hach Nitrate Test Kit to be more reliable than the Merckoquant test strips above 8.1 mmol/l NO_3. Wehrmann et al. (1982) brought stem segments (0.5 cm long) of 30 wheat stalks into reaction with 2-3 drops each of solution of 1 g diphenylamine in 100 ml concentrated H_2SO_4. Plant segments and reagent were pressed between two glass plates. Wollring and Köhler (1989) used the same method for petioles of petunia. Concentrations above 64.5 mmol/l NO_3 cannot be measured with this method. Steam destillation has then to be used.

Scheunemann and Paschold (1989) analysed the NO_3 content with the nitrate selective electrode in the sample with copper sulphate.

The NO_3 determination by diphenylamine is very old. It was already used by Hoffer in 1930 (cited by Wickstrom, 1967). Rauschkolb et al (1974) used a mixture of $BaSO_4$, $MnSO_4$, Zn, citric acid, sulfanic acid and naphatylamine and measured colour visually. This mixture is mixed with plant sap. Schaefer (1986) improved the visual estimation of the colour of the Merckoquant NO_3 test strip four times by using a reflectometer to measure the colour of the strip. This meter can be used between 8-34°C. The test strips were compared with ion-chromatography (Dionex). The test strips gave lower contents in sap of sunflower than the ion-chromotography. For example the Dionex gave 15 mM NO_3 and Merckoquant 6 mM NO_3 (60% lower). The reason was an interaction of the chemical in the Merckoquant test strip with the sap.

Schulz and Marschner (1987) used amino-N test for assessing N status of plants. The principle is that ninhydrin reacts with amino acids and with primary and secondary aliphatic amines, whereby coloured reaction products are formed. Ninhydron reacts especially with glutamine acid, glutamine, alanine, leucine, and less with asparagine acid, and not with asparagine. When wheat takes up NO_3 then glutamine acid and alanin are the dominant amino acids. Absorption of NH_4 leads to predominance of alanin. If sap is diluted 10 times the chlorophyl does not interfere much with the colour measurement. The colour can be determined by a reflectometer.

2.3.2. Other elements

For P there is no test strip of Merckoquant. Burns (1988) used a Mo blue related Hach orthophosphate test strip using one drop of the sap. The method is comparable with NO_3. The reaction is from a powder (PhosVer III). The blue color has to be compared with standards. The reagents for the Mo blue method is stable for 1 day. P can be determined by spectrophotometry with ammonium molybdate, antimony potassium-tartrate, sulfuric acid and ascorbic acid. Correlation between this method and Hach method was good. With small volumes the vanadomolydate method has the disadvantage to underestimate P content. Molybdynum blue does not have this problem (Burns and Hutsby, 1986).

Burns and Hutsby (1981) compared the P-colorometric method by vanadomolybdate complex (150 ul sap) and the molybdynum blue complex (50 ul). The correlation was good, the vanadomolybdate tended to be slightly higher. This was caused by slight brown colouration often shown by the sap samples of deficient plants. The molybdynum blue method is recommended. Hunt (1981, Annual Report, National Vegetable Research Station, Wellesbourne, UK) found for the P-molybdenum blue method a coefficient of variation of 1.75 and 2.64% and for K-flamephotometry of 2.84 and 1.53% for potato and bean, respectively.

Burns and Hutsby (1984, Annual Report National Vegetable Research Station) found that the Merckoquant K test strip overestimated the K concentrations in the sap below 20 mM. Errors could be introduced by putrescine and agmatine in the plant, which is known to occur under K deficient conditions. At severe K deficiency enhanced colouration of the sap could interfere. K could be determined between 25-50 mM. NH_4 > 5.5 mM interfered with K-Merckoquant. Vielemeyer and Weissert (1990b) analysed P (PO_4) by spectrophotometric vanadate-molybdate method (1:50 dilution of the press sap with distilled water), Ca and Mg was determined by atomic absorption spectrophotometry (1:100 dilute of the press sap by 0.1 M HCl). For the determination of Ca 0.1% Sr has to be added in the form of $SrCl_2$ as spectrophotometric buffer. Determination of micro-elements was not possible.

Syltie et al. (1972) used colour standard sheets for the determination of NO_3, P, K, Mg and Mn.

Burns found high correlation between K-Merckoquant and K-flame photometry at K contents > 15 mM. In lettuce trials K contents were between 0-50 mM. Concentrations < 15 mM could not be determined by Merckoquant test strips.

Bettin (1991) pressed fresh petioles of azalea. The sap was diluted and put on a filter paper which was mixed with a ninhydrin reagens, which reacts with organic N. The red colour is a measure for the

'amino-N' content. Taylor found total-N was not suitable, better was arginine. Because of the interference with flavonoïde it is simpler to determine alpha-amino-N. This holds for trees and Rosaceae.

Bar-Akiva (1984) used Na-acetatebuffer in H_2O_2 with 3,3 ,5,5 tetramethyl-benzidine (TMB). When the enzyme peroxidase is active then a blue colour is changed to the red colour. TMB-H_2 (colourless) + 2 H_2O_2 -> peroxidase -> TMB (red) + 2H_2O. In this way Fe-deficiency can be recognized and dinstinguished from Mn-deficiency. The enzyme peroxidase contains Fe. The red colour is found when there is enough Fe in the plant. It is also possible to use 3-amino-9-ethylcarbazol (AEC) for this purpose.

Melsted (1950, cited in Wickstrom, 1967) used dipicrylamine for K quicktest.

Coltman and Riede (1992) used K teststrips of Machery-Nagel, Duren, Germany. These strips gave good results between 5.1 and 25.6 mM. The resolution of the strips was < 2.6 mM.

Smith (1987) measured plant nutrients in the sap using the extracts of sap like nutrient solutions and used the following analytical techniques:
ion specific electrode: NO_3
flame photometry: K, Na
colorometry: P, B
atomic absorption spectrophotometry: Fe, Cu, Zn, Mn (acidified sap)
Ca, Mg (non acidified sap)

Routchenko (1967) determined in sap: NO_3, NH_4, H_2PO_4, SO_4, Cl, K, Ca, Mg, Na, amino-N, proteine-N, total soluble-N, total-S, glucide-P, nucleïneacid-P, alcohol soluble-P, total-P, alcohol soluble-N. For these 18 analyses 6 extractions and three ashings were needed.

De Bes and van Dijk (1979) found that addition of acid was not required for SO_4 determination by autoanalyzer. Dark coloured sap samples inferfered with B determination.

Hunt and Moore (1982) determined Mn directly in the sap by atomic absorption spectrophotometry, without interference with other elements.

3. FACTORS INFLUENCING SAP CONTENTS

3.1. Nutrition

Wollring and Köhler (1989) compared NO_3 content in sap of petunia at three N levels. The N supply was as NH_4NO_3 and urea in equal amounts. NH_4 is nitrified and absorbed as NO_3. Optimal fertilization for quality of petunia was 33 mg N per pot, for maximum weight of the plant the best rate was 66 mg N per pot. N supply increased N contents in the sap.

Table 7. Effect of N fertilization on NO_3 in sap of petunia (Wollring and Köhler, 1989)

N-supply mg N/pot	NO_3 - sap content, mM average	range
16.5	14.5	3.2 - 69.4
33.0	50.0	19.4 - 79.0
66.0	108.0	67.7 - 127.0

Pettinger (1931) found a good correlation between N in the soil and N in sap of stem of maize. The relationship between N fertilization and N in sap however was not good. The correlation between K in the soil and K in sap was good, but this was not the case with P.

Bettin (1991) found a linear relationship between N in substrate and amino-N in press sap of azalea. There was no influence of either NO_3 or NH_4 supply on the amino-N; both forms of N gave the same reaction. Azalea with low amino-N had also visual chlorosis and plants with high amino-N had a dark green colour. So visual observation gave also an idea about N fertilization. It was concluded that for azalea amino-N content did not give a better indication of N status of the plant than visual observation. Fertilization with other elements did not interfere with amino-N content.

Coltman (1987b) measured NO_3 in petioles of tomato in relation to N fertilization. Correlation coefficients between these factors are given in Table 8.

Table 8. Correlation coefficients between N fertilization and NO_3 in petioles of tomato (Coltman, 1987b).

	Correlation coefficients				
Week after planting	2	4	6	8	12
	0.19	0.66	0.83	0.95	0.74

Only 8 weeks after planting correlation coefficient was good. In his experiments N-fertilization was applied continuously. He found lower optimal NO_3 contents in sap than other workers, who probably did not use continous fertilization. Coltman concluded that with continous fertilization optimal NO_3 levels in sap could be lower than with uncontinous fertilization, although this theory was not tested in his

trials. He stated that the N-form has also an influence. The NO_3 in the sap increased with time.

Alt and Füll (1988) found an increased NO_3 content in midribs of lettuce with the increased N supply (total 6 levels, 0-200 kg N/ha). Optimum production was found at 100 kg N/ha, corresponding with 520 mg/l NO_3-N. In midribs of old leaves the NO_3 content is twice than in the entire plant. So, optimum level in the entire plant is 280 mg/l NO_3-N. They concluded that is was possible to grow lettuce with NO_3 levels in the lettuce leaves below the desired level for human consumption. This trial was conducted in summer but in winter under poor light conditions the results are likely to be different. Their conclusion was that it is possible to use the midrib of old lettuce leaves for estimating the optimum N supply of lettuce plants. Results of their trial are given in Figure 3.

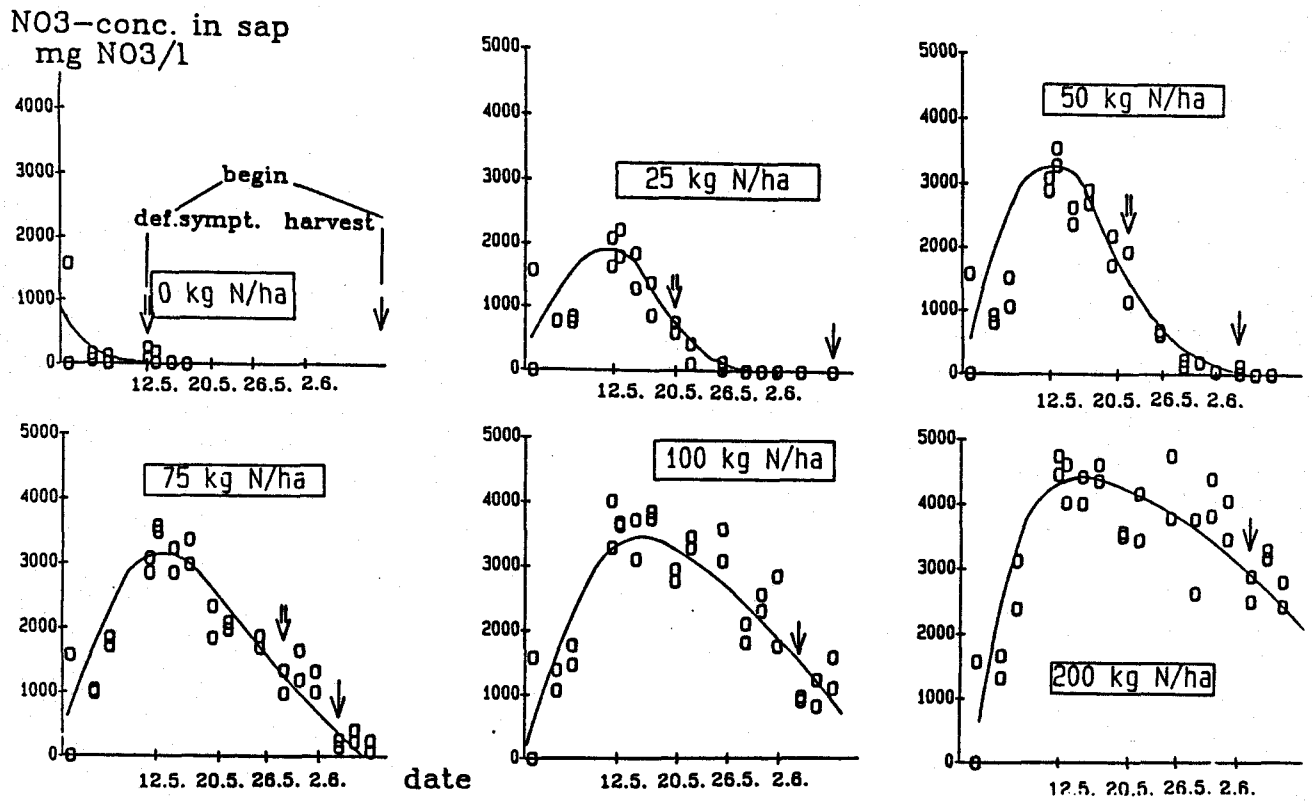


Fig. 3. Influence of N supply on the course of nitrate content in sap of midribs of lettuce leaves (field trial; Alt und Füll, 1988).

Hernando and Cadahia (1973) found effects of N, P and K fertilization on the levels in sap of tomato petioles (Table 9). After nutrient deficiency an application of N, P and K, restored the NO_3, P and K contents in the plant.

Table 9. Effect of fertilization on petiole sap contents of tomato (Hernando and Cadahia, 1973).

	NO_3-N mg/l	P mg/l	K mg/l
normal fertilization	475	65	4670
deficiency	222	44	3740
deficiency, but once a dosis of N,P,K	406	66	4530

Prasad and Spiers (1985) compared total-N, NO_3 soluble in acetic acid, NO_3 in sap with N fertilization of tomato. All three plant contents gave good relationships with production. Only for NO_3 in sap 11 weeks after planting there was no correlation with production. For N-total there was a good correlation.

Schenk (1988) investigated the relationship between N fertilization of Saintpaulia and Cyclamen under NO_3: NH_4 ratios in the liquid feeding supplied to the plant of 3:1, 2:2 and 1:3. Potting soil was analyzed as well. Results are given in Figure 4. N-fertilization rate gave a better correlation with NO_3 content in the sap than nitrate content of the potting soil. On 30 days after planting sap NO_3 contents (not shown) were higher than on 59 days after planting. For example at 60 mg/l N fertilization plant sap nitrate content decreased from 900-1100, 450, to 110 mg/l NO_3-N at 30, 59, 95 days after potting, respectively. Optimal growth was found at 88 mg/l N in the liquid feed.

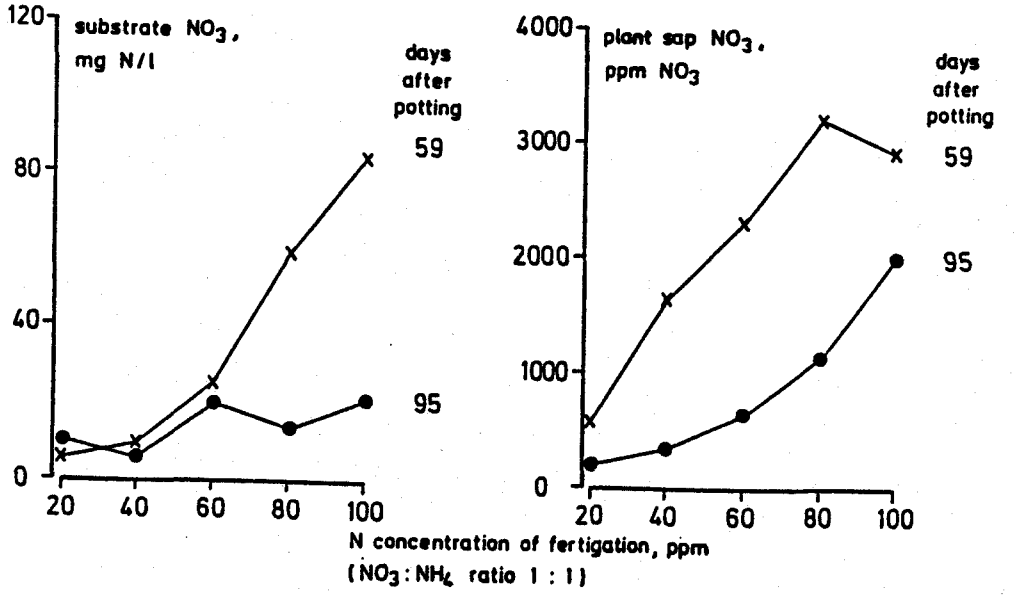


Fig. 4. Influence of N-concentration of fertigation on substrate and plant sap nitrate of Cyclamen pers. at different times after potting (Schenk, 1988).

In the 'Forschungsanstalt für Weinbau, Gartenbau, Getränketechnologie und Landespflege' in Geisenheim am Rhein, Germany, different experiments were conducted with lettuce, cauliflower, tomato, cellery and pot plants. (Annual reports of 1977 to 1982). This resulted in a scheme for N status (Table 10).

Table 10. Scheme for N-status of plants

Group	NO_3-N , mg/l	N status
I	0-43	very low
II	45-147	low
III	149-396	middle
IV	398-848	high
V	850-1130	very high
VI	>1130	too high

Adams (1982) related K in peat substrate to K in petiole sap of cucumber (Figure 5). At low levels there was a good relationship, but at high K levels in the peat the K sap content did not increase; it was about 4000 mg/l K.

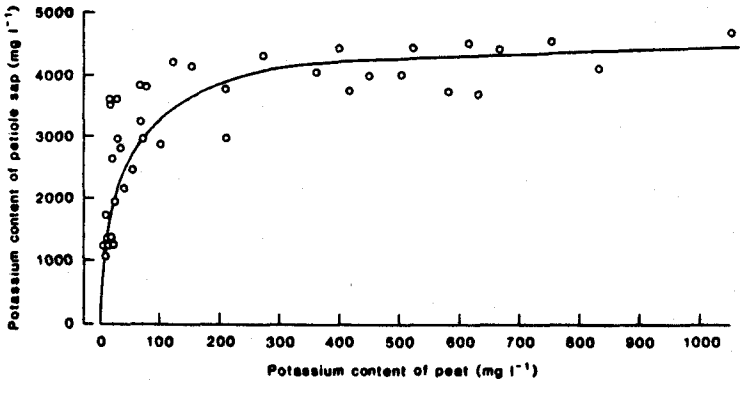


Fig. 5. Relation between the potassium contents of the sap pressed from the petioles and the water-soluble + exchangeable potassium in the peat (Adams, 1982).

Rahimi and Schropp (1984) compared the relationship between total Zn and Zn in sap to Zn in nutrient solution (Table 11). Sap Zn content gave a better indication of the Zn status of the plant than total Zn content.

Table 11. Effect of Zn on contents in the plant (Rahimi and Schropp, 1984).

Zn in nutrient solution ug/l	Plant weight g	Zn total mg/kg DM	Zn sap mg/l
Millet			
0	2	13.9	0.56
5	10	14.0	0.90
10	15	12.2	1.20
50	20	22.2	1.75
100	21	38.4	2.95
1000	6	250.0	7.65
Sugarbeet			
		after 30 days	
0	1	8.0	0.57
5	1	17.1	0.72
15	4	20.3	1.08
50	4	65.2	2.40
100	4	120.0	5.00
	after 40 days		
0	4	10.5	0.60
5	10	10.5	0.58
15	11	18.1	0.94
50	10	45.1	2.00
100	10	73.9	3.80
Tobacco			
	after 4 weeks		
0	5	12.0	0.37
5	12	11.7	0.52
10	15	10.5	0.60
50	20	23.0	1.20
100	28	35.7	2.00
1000	14	760.0	9.00
	after 6 weeks		
0	8	13.5	0.39
5	30	12.2	0.58
10	60	11.7	0.64
50	79	17.0	1.12
100	93	23.6	1.90
1000	50	465.0	7.00

Burns (1992a) found that after omitting N or P or K, the NO_3, P and K contents in sap decreased very fast. So there was a direct reaction of supply to the roots. He concluded that sap contents are good to predict fertilization level. It was found that the youngest leaves of lettuce react faster than the oldest. So youngest leaves are more suitable than oldest leaves.

In addition antagonisms between nutrients can be detected by sap analysis. For lettuce Burns (1986 and 1988) demonstrated the antagonism of K and Na. K deficiency is found when K sap content is below 70 mM in

old leaves and 34 mM in young leaves in absence of Na in the feeding. If Na is added to the nutrient solution K deficiency is found at K sap content below 40 mM in old leaves and 18 mM in young leaves. Also the antagonism between NO_3 and Cl has been shown, especially in fast growing plants (Hernando and Cadahía, 1973; Table 12). When NO_3 is high the Cl in sap is lower than with low NO_3.

Table 12. NO_3/Cl antagonism in tomato petiole sap (Hernando and Cadahia, 1973).

Sap content	Treatment	
	high Cl/low NO_3	high Cl/high NO_3
NO_3-N mg/l	344	2944
Cl mg/l	4430	1100

Coltman and Riede (1992) found a good relationship between K in the nutrient solution and K in petiole sap of tomato (Figure 6).

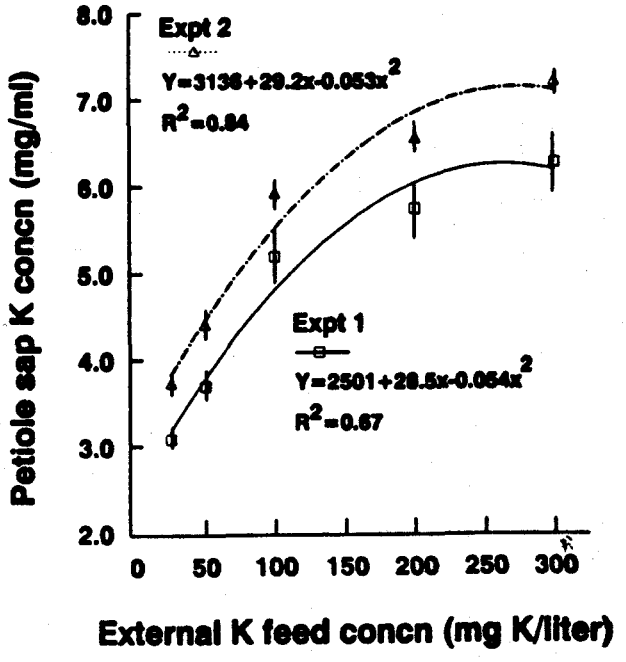


Fig. 6. Response of petiole sap K concentrations to external-feed K concentrations in two experiments (Coltman and Riede, 1992).

Alcarez et al. (1982) detected the following antagonistic effects:
tomato and sweet pepper Cl - NO_3;
tomato and sweet pepper Cl + SO_4 - NO_3;
sweet pepper SO_4 - NO_3.

For tomato no antagonistic effect was found between SO_4 and NO_3. Voogt (1982) demonstrated antagonistic effects between K, Ca and Mg (Table 13). The elements counteracts, especially between Mg and Ca.

Table 13. Effect of K/Ca/Mg levels in the nutrient solution on K, Ca, Mg contents in laminae and petiole sap. Contents in mmol/l (Voogt,1982).

Nutrient solution			Cucumber laminae			Cucumber petiole		
K	Ca	Mg	K	Ca	Mg	K	Ca	Mg
6.5	3.5	0.5	90	21.4	6.4	105	21.2	2.7
5.5	3.5	1.0	87	19.4	12.5	98	19.8	6.2
4.5	3.5	1.5	77	21.0	18.8	95	20.7	6.1
8.2	2.5	0.6	106	12.4	8.1	116	11.8	2.4
7.0	2.5	1.3	94	12.3	17.8	111	11.6	5.9
5.7	2.5	1.9	90	14.0	27.6	110	11.1	8.0

Nutrient solution			Eggplant leave			Eggplant petiole		
K	Ca	Mg	K	Ca	Mg	K	Ca	Mg
6.5	3.5	0.5	214	21.1	9.6	210	11.5	6.2
5.5	3.5	1.0	207	17.2	10.8	212	11.2	11.5
4.5	3.5	1.5	204	16.5	13.1	196	10.7	16.4
8.2	2.5	0.6	218	10.2	10.2	212	6.0	5.4
7.0	2.5	1.3	202	9.6	16.4	200	6.2	20.0
5.7	2.5	1.9	198	11.1	22.2	206	5.4	24.7

It is also possible to see salt damage when Na levels are > 1000 mg/l Na (Hernando and Cadahia, 1973). Normally Na content is 500- 700 mg/l. De Kreij (1989 and 1990) found a good relationship between B in root environment and B in sap of young fully grown leaves (Table 14).

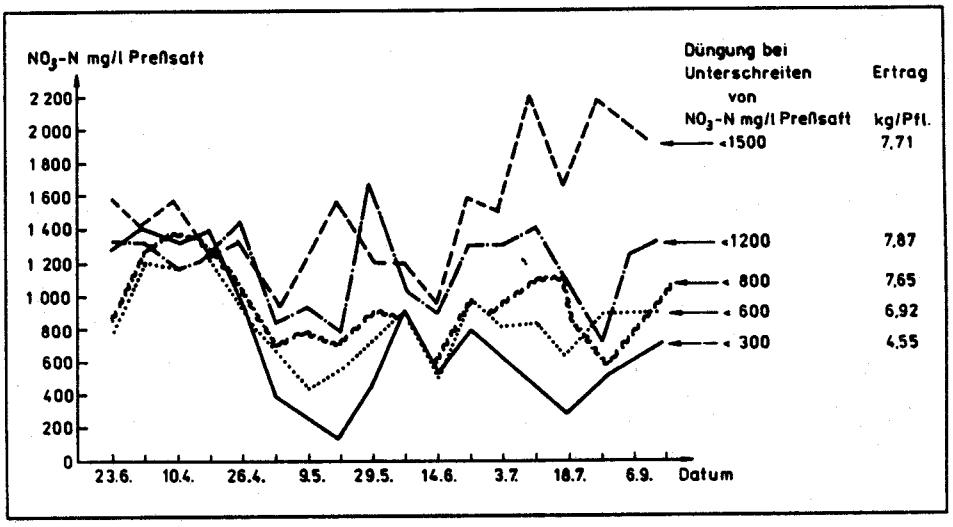
Table 14. Boron in root environment and in sap of young, fully grown and old leaves (De Kreij, 1989 and 1990).

B in root environment umol/l	Boron in sap Gerbera young umol/l	old umol/l	B in root environment umol/l	B in sap of carnation umol/l
10	145	156	12	488
20	158	162	32	766
50	204	306	71	823
100	206	846	116	863
			203	1211
			278	2443

Burns and Hutsby (1981) omitted P with lettuce in sand culture. After 11 days sap of young to middle aged leaves contained 30 mg/l P. The control with normal P supply contained 100 mg/l P. Of the first

treatment the P content decreased during 11 and 21 days after the omission of P, and stayed constant at 10 mg/l P. Omitting K leaded to K content in sap of young leaves of 0 mM. In the control it was 70 mM.

Drews and Fischer (1992) conducted a trial with tomato where they fertilized when sap contents were below 300, 600, 800, 1200 and 1500 mg/l NO_3-N respectively. Results are given in Fig. 7. The relationship between NO_3 and production in Fig. 8. 1000 mg/l NO_3-N gave the highest production. In another trial K fertilization started at K content in sap of tomato of 2500, 3000, 4000 and 5000 mg/l K. Results are given in Fig. 9. Maximum production was found at 4000 to 5000 mg/l K (Fig. 10).



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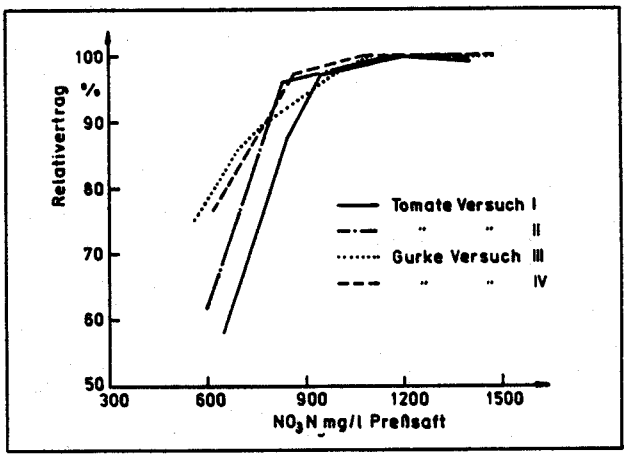

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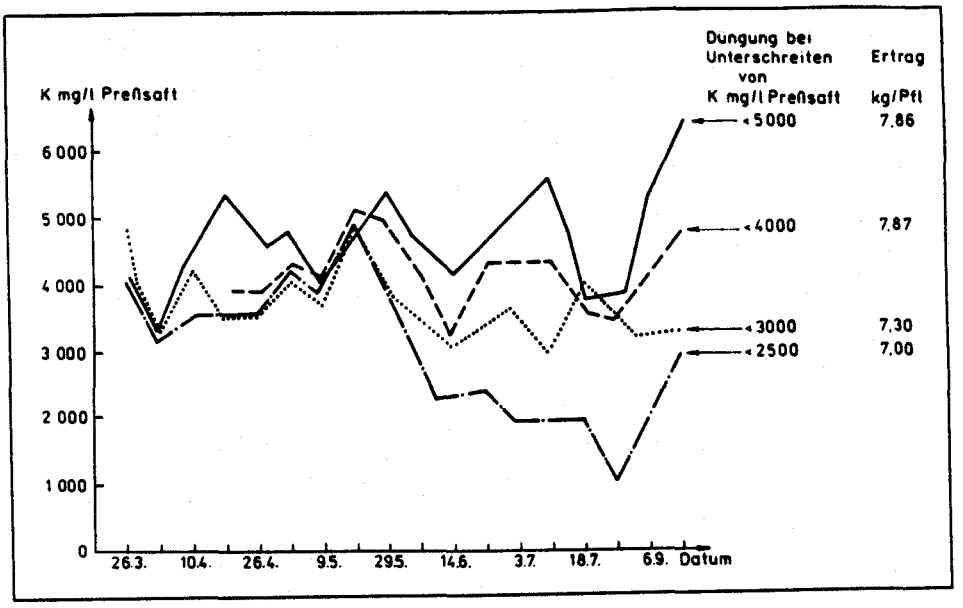

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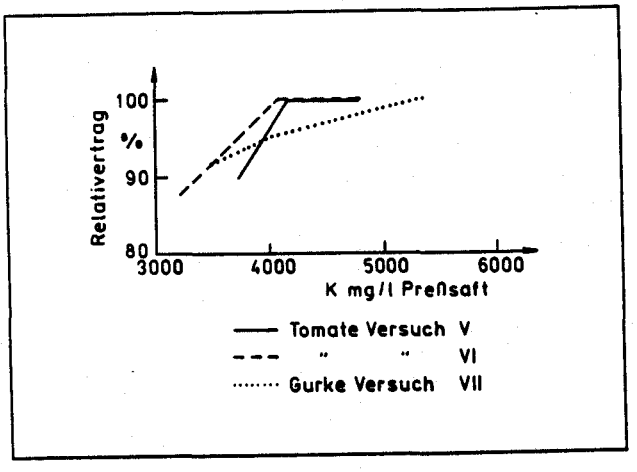

10.

Fig. 7-10. Yield and NO_3-N in press sap of tomato (Fig. 7), of K of tomato (Fig. 8), and of tomato and cucumber (Fig. 9-10; Drews and Fischer, 1992).

3.2 Plant part

It is important to know the variation between plant parts in order to select the best part of the plant for analysis. In France Morard took the petioles of plants, and not the laminae, because the latter are storage organs for nutrients and not sensitive to nutrient changes in plants. Similarly side shoots have the same advantage as petioles.

Much research was done by Burns and Hutsby (1981, 1984, 1986, 1991) and Burns (1988, 1990, 1992) to find out which is the best leaf to sample. They omitted K fertilization of lettuce grown in coarse sand. In the control K fertilization was not stopped. In Table 15 the time it took for K content in sap to get lower than in the control is given. Also in this table it is shown how much time it took to get an increase of K in sap after K fertilization was started again.

Table 15. Reaction time (in days) of K in sap after omitting K fertilization and starting it again, in relation to a control with continous fertilization.

	Reaction time omitting K days	Reaction time after starting K again days
young immature leaf	5	4 - 10
middle leaf	9	7 - 20
old leaf	22	*)

*) In old leaves K content never reached the control.

Young immature leaf gave the fastest reaction on omitting K and starting fertilization again. Young leaves are more sensitive than old leaves. In Figure 11 the K contents of leaves of lettuce are shown. The control had a supply of K and Na. In all the treatments it was found that the oldest leaves had higher K than younger leaves.

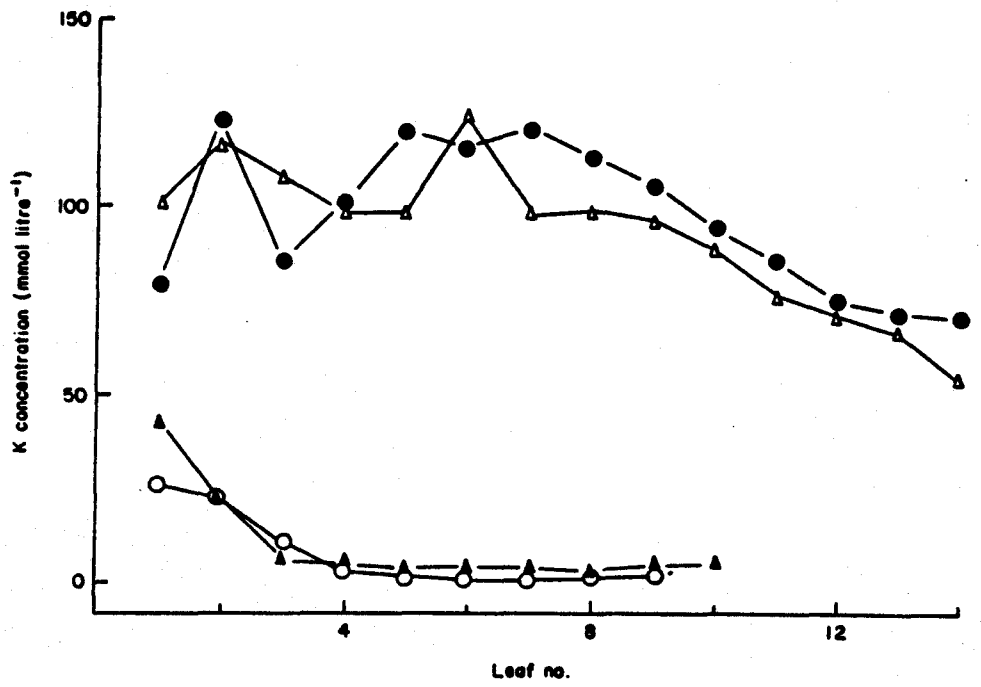

Fig. 11. Concentrations of K in petiole sap from invidual leaves of lettuce plants. ● = Control K + Na fertilization; 0= zero-K; Δ = zero-Na; ▲ = zero (K+Na). Leaf 1 is the oldest leaf sampled.

It was also found that old leaves had higher NO_3, but lower P than young lettuce leaves. Figure 12 gives the distribution of NO_3, K and P in lettuce plant. Omitting fertilization was also investigated for N and P. For these elements it was also found, that the youngest leaves react faster than the oldest leaves. Their conclusion is, that it is best to take the youngest leaves for determing N, P and K status of the plant.

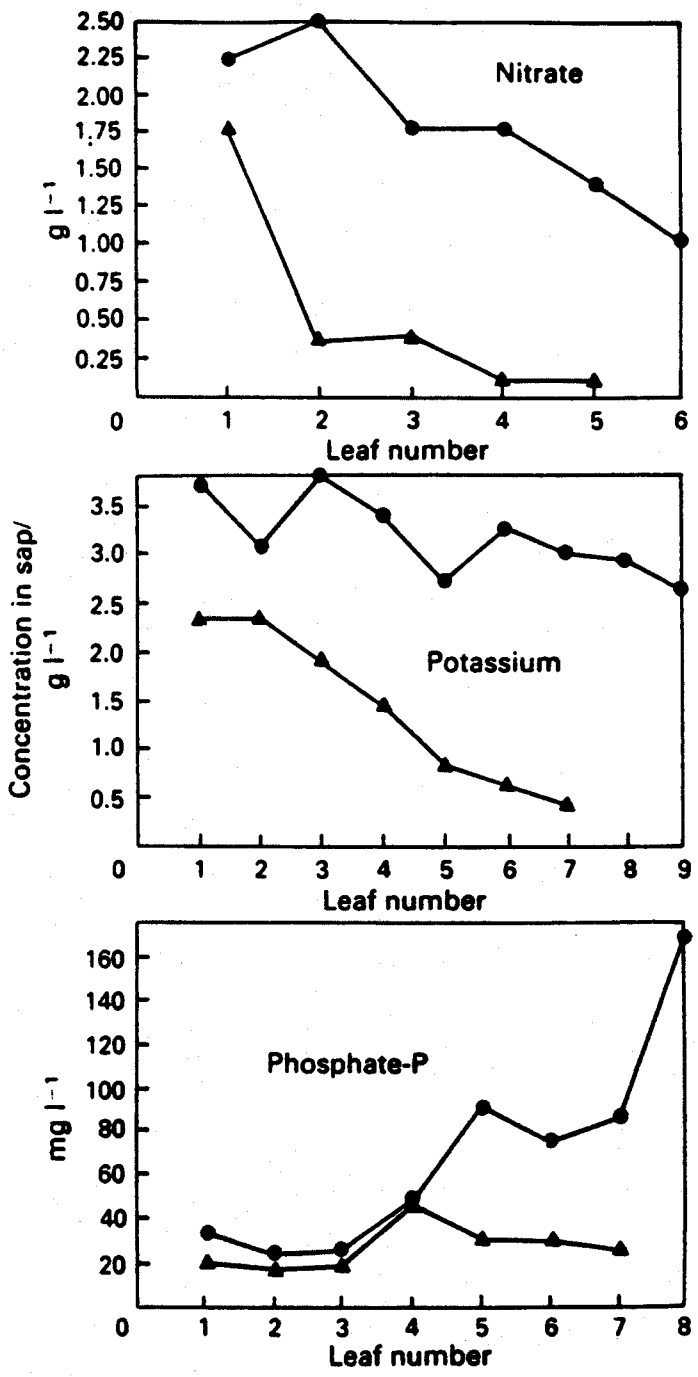

Fig. 12. Distributions of nitrate, potassium and phosphate between individual leaves of non-deficient plants (●) and deficient plants (▲). Leaf 1 is the oldest lead sampled

Vielemeyer and Weissert (1990a) did an experiment to find out which petiole (young, middle or old leave) was the best to sample. They grew tomato in complete nutrient solution. For 2 weeks (with Mg 4 weeks) a certain element was withheld. After supplying that element again the petioles of young, middle and old leaves were sampled on 0, 3, 7 and 14 days after restarting the fertilization.

Table 16. Contents in petioles of tomato. Nutrients were withholded for 2 weeks. After restarting fertilization (Day 0) and on Day 7 petioles were sampled. C=control; W=withholded (Vielemeyer and Weissert, 1990a).

	young				middle				old			
	Day 0		Day 7		Day 0		Day 7		Day 0		Day 7	
	C	W	C	W	C	W	C	W	C	W	C	W
NO_3-N,mg/l	800	150	1100	800	1300	400	1300	700	2800	1800	2600	2000
Rel	100	19	100	73	100	31	100	54	100	64	100	77
PO_4-P,mg/l	450	200	400	400	700	300	800	400	1000	8000	1000	700
Rel	100	44	100	100	100	43	100	50	100	80	100	70
K,mg/l	4500	2500	6000	5500	5700	3400	6000	4000	5000	4000	5200	4400
Rel	100	56	100	92	100	60	100	67	100	80	100	85
Ca,mg/l	200	0	180	150	1080	480	1000	300	2100	1600	1800	1400
Rel	100	0	100	83	100	44	100	30	100	76	100	78
Mg,mg/l	200	100	200	130	500	180	600	200	1430	900	1380	1000
Rel	100	50	100	65	100	36	100	33	100	63	100	72

The withdrawal of nutrients led to a larger decrease of nutrient content in the young petioles than in the middle or old for all the elements. Except for Mg the middle petioles react quicker than the young or old petioles. After renewed supply of the nutrients with PO_4-P and K in the petioles of young leaves the element contents were the same as in the control after 3 days, for NO_3-N, Ca and Mg after 14, 7 and 14 days, respectively. In middle and old petioles the element contents did not reach the control. It was concluded that the young leaves are the best for sampling.

Smith (1988) sampled petioles of tomato. Results are given in Table 17. K, Na, Fe are quite stable. NO_3-N, P, Ca, Mg, Zn, Mn and B increase from higher to lower leaves. Cu decrease from higher to lower leaves.

Maynard et al. (1976) found that NO_3 concentration are higher in older leaf parts than in younger and higher in petioles than in laminae.

Table 17. Contents in petioles of leaf 9-13 from the top of tomato (Smith, 1988).

	Sap contents in leave 9 and or relative concentrations to leaf 9 Leaf number from the top				
	9	10	11	12	13
	mg/l	%	%	%	%
NO_3-N	470	100	103	132	146	154
P	415	100	126	127	136	146
K	5160	100	99	101	101	100
Ca	820	100	100	104	111	125
Mg	259	100	105	112	115	136
Na	170	100	88	100	100	100
Fe	1.55	100	106	97	97	103
Cu	1.95	100	56	52	59	49
Zn	2.70	100	119	119	146	146
Mn	5.65	100	96	109	114	118
B	2.05	100	100	137	137	129

Lindhauer et al. (1990) grew sugarbeet for 63 days on low levels of K or Na (0.5 mM) or high levels (4.5 mM). Different plant parts were pressed and K, Na, Mg and Cl in the sap were determined. Results are given in Table 18.

Table 18. K, Na, Mg and Cl in sap of different plant parts of sugarbeet grown at low (0.5 mM) K or Na or high K or Na (4.5 mM). Sap contents in mM (Lindhauer et al., 1990)

treatment	high K low Na				low K high Na				low K low Na			
leafpart	K	Na	Mg	Cl	K	Na	Mg	Cl	K	Na	Mg	Cl
Laminae												
old	351	75	55	71	95	298	47	38	102	166	34	21
middle	281	52	42	56	111	234	40	29	114	111	22	16
young	219	41	26	46	111	160	27	20	102	53	17	13
Petioles												
old	315	44	24	209	47	289	20	170	37	114	39	100
middle	222	28	19	149	55	199	12	121	55	55	30	70
young	163	17	15	88	61	128	9	68	66	24	18	35

In old plants K, Na, Mg, Cl are higher than in young plant parts, except K is lower in older plant parts when K nutrition is low. Ca in sap were very low, although in dry matter the Ca content was 125-475 mmol/kg DM.

Morard et al. (1987) found in petioles of very young growing leaf of strawberry lower N, Ca and Mg levels than in petioles of young, fully grown leaves. Therefore he recommended to sample petioles of young, fully grown leaves. In young leaves coefficient of variatition was lower than in old leaves (Burns, 1986).

Bettin (1991) found in young stem of azalea 346 mg/l amino-N, in middle aged stems 468 mg/l and in old leaves 456 mg/l.

Taylor (1971) determined which plant part of peach was best correlated with N fertilization. The most sensitive parameter was arginine but it was difficult to determine because of colour interference from flavonoïden. Therefore it was easier to determine alpha-amino N. The best relationship with fertilization was found for the roots, then leaves and flowerbuds. This holds for all trees and Rosaceae (for example rose). In these crops NO_3 is reduced to amino acid in the roots. NO_3 in leaves or petioles can not be used to assess N fertilization levels. Therefore amino acids have to be determined.

Alt and Heitkamp (1984) found in midrib of old lettuce leaves twice the NO_3 content as in the whole plant. So it is easy to find the NO_3 content of the lettuce by determining sap NO_3 content in midrib and dividing that by two. Another example of this feature is given by Alt and Füll (1988) in Table 19.

Table 19. NO_3-N in sap of lettuce (Alt and Füll, 1988).

Plant part	NO_3-N mg/l
middle leaf	1659
young yellow-green leaf	2016
almost mature green leaf	3314
tallest leaf	4618
old, partly small and detiorated leaf	6983

Schulz and Marschner (1987) found in wheat the following differences in amino-N: stem < old leaf < young leaf

Rauschkolb et al. (1974) found in stem of maize higher NO_3 than in midrib.

Hernando et al. (1982) took for routine testing of tomato plants the petiole of the leaf just above and under the flowering truss. To support this idea an experiment was conducted. They sampled very young growing leaves (second leave to the top from the highest flowering truss), fully grown young leave (at the first flowering truss) and matured leave (old leaves, second leave downwards from the first flowering truss). Results are given in Figure 13. In old leaves NO_3, Ca and Mg was higher and P was lower than in young leaves. In old leaves also K was lower than in young, which contradicts with most of the earlier findings between old and young leaves. The amino-N and proteine-N and P do not differ very much between the different plant parts. In old leaves NO_3 is very high. According to Hernando it was concluded that old leaves are not a good indicator for N status of the plant.

Martinez-Canadas et al. (1985) took 10 samples of petioles of pepper and analyzed it using four replications. Coefficient of variation (c.v.) was determined (Table 20).

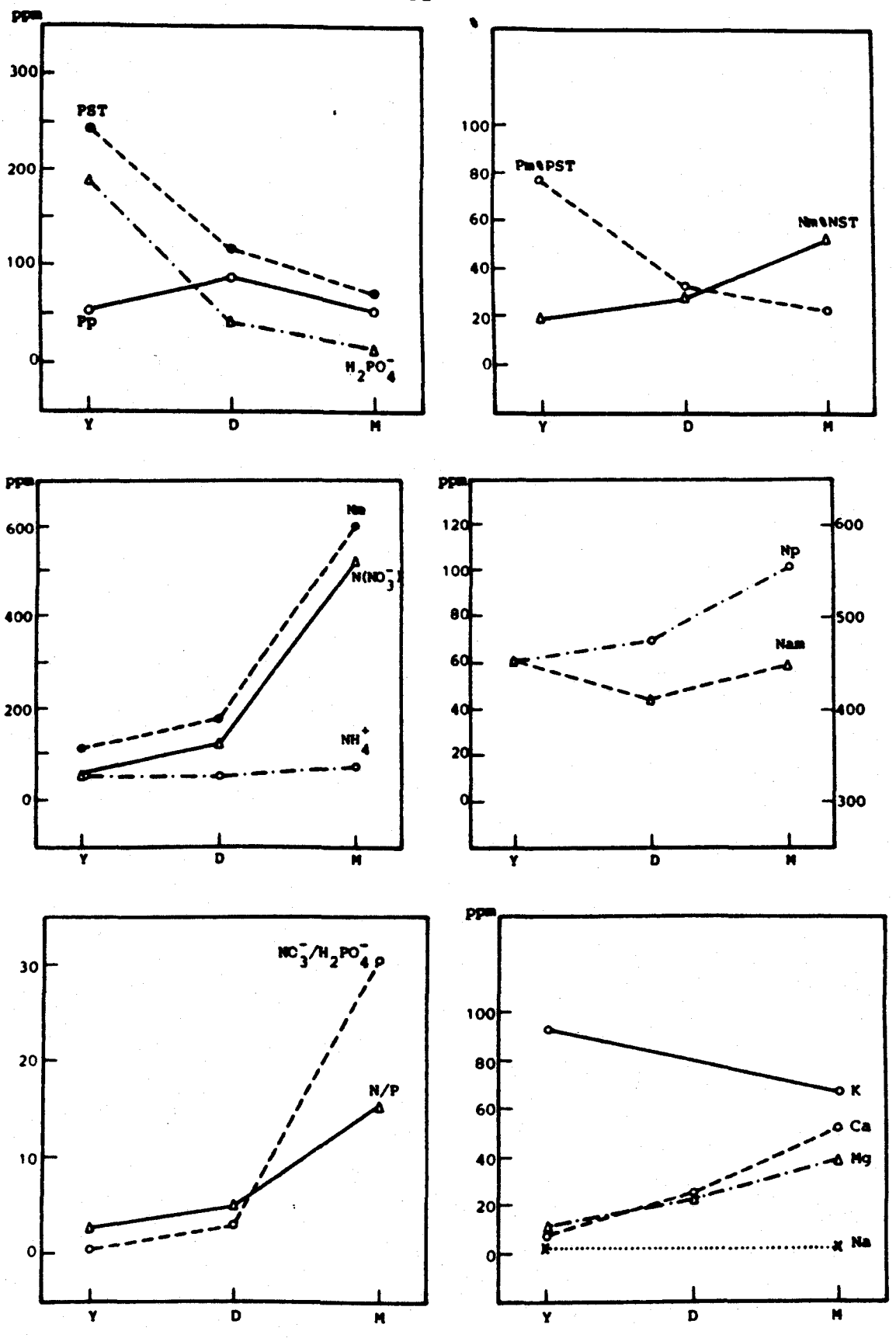

Fig. 13. Contents in petioles of tomato; Y = young growing leaf; D = fully grown young leaf; M = fully grown matured leaf N_m = mineral N; N_{am} = aminoacids-N; N_p = protein-N; P_p = protein-P; P_m = mineral-P; PST = total P; NST = total-N (Hernando et al.,1982).

Table 20. Mean and coefficient of variation of sap content of petioles of sweet pepper (Martinez-Canadas et al., 1985).

Element	Top		Middle		Old	
	Mean mM	c.v. %	Mean mM	c.v. %	Mean mM	c.v. %
NO_3	110	13	145	7	180	11
H_2PO_4	2	13	2	1	1	16
Cl	60	11	81	6	95	6
SO_4	11	10	25	5	20	8
K	234	10	296	4	322	8
Ca	0.3	19	0.2	10	0.1	20
Mg	35	13	27	7	23	10
Na	10	18	10	9	8	11

In old leaves NO_3, Cl, K were higher and Mg was lower than in young leaves. The coefficient of variation was the lowest in petioles of young leaves. So Martinez-Canadas et al (1985) concluded that the petioles of young leaves are the best part to sample.

Sonneveld (1980) found in petioles of old leaves of tomato more Mg, SO_4, Mn and Zn than in young leaves. Other elements were not different. For cucumber only Ca was higher in old leaves.

Scaife and Stevens (1977) found in young petioles of cabbage 55 mg/l NO_3-N, for mature petioles 118 and for the oldest 26 mg/l. This pattern was confirmed again by Scaife and Stevens (1983).

3.3. Sampling time

Many researchers have concluded that time of the day hardly influenced the results (Scaife et al., 1983; Drews and Fischer, 1992; Bettin, 1991; Scaife, 1979; Coltman, 1987; Smith, 1987; Hernando and Cadahia, 1973; Vielemeyer and Weissert, 1990).

Coltman (1987) sampled tomato on 8.30, 11.30 and 14.30 hours and determined NO_3 in petioles for 6 days. On individual days there was an influence of sampling time but averaged over the 6 days, the sampling time had no influence.

Scaife (1979) found that on sunny days in the mid-afternoon plants with low N status the NO_3 content was lower than in the morning. However this influence was small.

Azuara et al. (1982) in Spain sampled entire young tomato plants on 0, 2, 4, 6 and 10 hours after sunrise in a greenhouse. They analyzed NO_3, amino-N, protein-N, NH_4, H_2PO_4, protein-P, SO_4, Na, K, Ca, Mg and sugars. Sampling time influenced NO_3, protein-P and sugars. NO_3-N was in the morning 500 mg/l and 10 hours after sunrise it was 300 mg/l. Protein-P decreased from 260 mg/l P at sunrise to 160 mg/l P at 10 h after sunrise. Sugars increased from 7000 mg/l to 13000 mg/l between sunrise and 10 h after sunrise. Other elements were not influenced by sampling time.

Hernando et al. (1987) sampled every three hours between 5.00 h and 20.00 h petioles of tomato and determined NO_3, amino-N, protein-N, P and

K. On the middle of the day (14.00 h) NO_3 was 20% lower than in the morning and amino-N was 12% higher. Protein-N, P and K were constant during the day.

Scaife and Stevens (1977) sampled between 6 a.m. and 8 p.m. every 2 hours petioles of large mature leaves of carrots and cabbages. The NO_3 content tends to fall to about half their morning values by 4 p.m. and then rose again as night approached. This is because nitrate in leaves is being rapidly converted to protein during the afternoon (Scaife and Stevens, 1983). They sampled petioles of cabbages on a clear sunny day (14 September 1977) every 2 hours from 6.00 h. until 20.00 h. No significant influence of sampling time could be found. This latter conclusion is difficult to understand in relation to their earlier data of 1977.

Vielemeyer und Weissert (1990) determined the influence of weather and variation over short period of time on the nutrient contents of petiole of cucumber. The time of sampling was 7.00 hour (Table 21). Although the weather changed: there was no effect on nutrient content.

Table 21. Contents in petiole of cucumber (Vielemeyer und Weissert, 1990).

Day	Temp. min/max	NO_3-N	PO_4-P	K	Ca	Mg
16/5	12/26	1550	220	5700	830	140
17/5	12/21	1500	220	5700	910	150
18/5	8/11					
19/5	9/16					
20/5	9/11	1550	230	5420	900	140
	n.s.	n.s.	n.s.	n.s.	n.s.	

Geyer und Marschner (1990) found decreasing NO_3 contents in leaf sheath from basal stem of maize over time. For example graded as high in leaf sheath of basal stem it was in 4-5 leaf stage 1100-1700 mg/l NO_3-N, but in silking stage 400-700 mg/l NO_3-N.

Smith (1988) sampled petioles of tomato mid-leaf from 8.30 a.m. to 4.00 p.m. at half hourly intervals. P, Mg, Zn and B had a difference between the highest and the lowest value of around 20-30%. Ca and Mg showed a 5% variation and NO_3-N, K, Na, Fe and Cu showed no consistent pattern through out the day. On a warm, sunny day the results were different. Fe increased from 1.8 mg/l at 1 p.m. to 6.8 mg/l at 4 p.m. B was also higher in the afternoon. Other contents fluctuated by about 30%

Gardner and Tucker (1967) noticed that NO_3 in petioles of cotton decreased in the growing season by a factor 10.

Hernando and Cadahia (1973) determined sap contents of petioles of tomato sampled several times of the day (Table 22).

Table 22. Sap contents of petioles of tomato (Hernando and Cadahia, 1973). Concentrations in mg/l

	Time						
	6.45	9.30	11.30	13.00	15.45	18.45	22.00
N (NO_3)	1125	1000	963	825	825	963	913
N (NH_4)	190	155	100	90	140	185	200
P (H_2PO_4)	78	88	80	79	76	80	90
S (SO_4)	78	66	56	50	51	74	55
K	7470	6625	6640	5965	6125	6135	6750
Ca	63	50	25	12	12	12	12
Mg	293	287	255	183	226	230	250
Na	59	55	55	44	70	56	54

Vielemeyer and Weissert (1990) found no significant effect of the sampling time (table 23).

Table 23. Sap contents at different sampling time (Vielemeyer and Weissert, 1990.

Sampling time.	Contents in petioles of cucumber, mg/l				
	NO_3-N	PO_4-P	K	Ca	Mg
7.00	1650	230	7060	900	150
11.30	1660	200	7090	940	150
16.00	1790	220	7300	980	160
	n.s.	n.s.	n.s.	n.s.	n.s.

Many researchers found a strong decrease of NO_3 content during the growing season.

Beringer and Hess (1979) reported a decrease of NO_3 content in winter wheat during the growth period from 903 to 135 mg/l NO_3-N. Optimum levels related to marketable yield of carrots, was a NO_3-N concentration of 1300 on 19 June, 9 on 8 July and 0 on 7 August (Scaife and Turner, 1980) Annual Report NVRS.

Scaife and Turner (1981, Annual Report NVRS) gave some other examples for optimum NO_3 concentration for different crops related to sampling time (Table 24). Scaife et al. (Annual Report NVRS 1983) reported for lettuce in greenhouses a critical concentration of 600 mg/l NO_3-N at emergence and 2400 mg/l NO_3-N at maturaty.

A lower NO_3 content for wheat over time was reported by Elliott et al.(1987), for kenaf by Lyons et al. (1991; Fig. 14), for cotton by Constable et al. (1991) for barley by Wollring (1983), for cabbage, lettuce, leek, onion and spinach by Scaife et al. (1983).

Table 24. Optimal NO_3-N concentration sampled on different dates (Scaife and Turner, 1981, Annual Report NVRS).

Spinach	Optimal NO_3-N mg/l	Lettuce	Optimal NO_3-N mg/l
28 May	2800	26 May	1600
9 June	1200	8 June	1800
17 June	1350	22 June	740
harvest	80	20 July	20
Onions		Leeks	
7 June	330	2 July	450
23 July	13	30 July	75
7 Sept	6	14 August	75

Coltman (1987a) found low NO_3 content in petiole of tomato at early bloom (39 days after planting; Figure 15). The reason for this dip in nitrate content is not clear. So it is difficult to use NO_3 content for fertilization scheme.

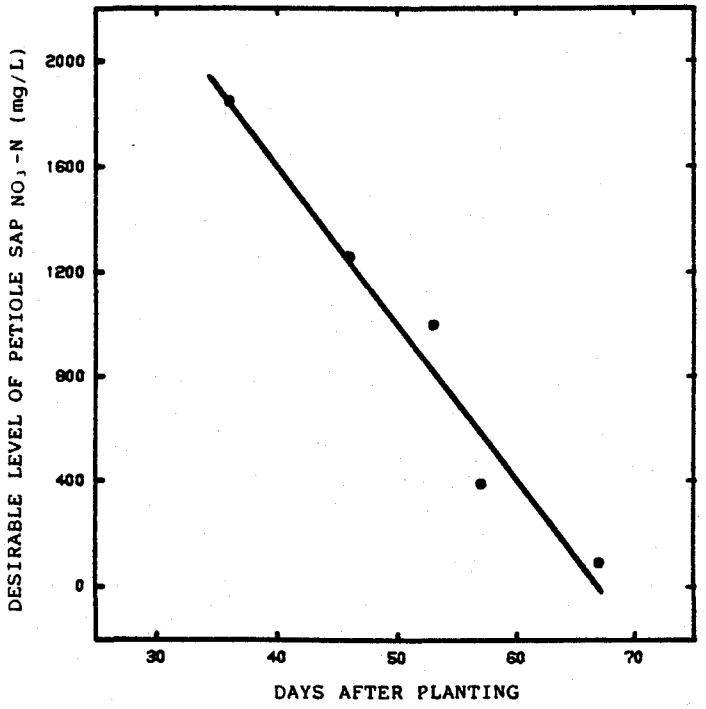

Fig. 14. The change in desirabele levels of petiole sap NO_3-N for kenaf with time (Lyons et al., 1991).

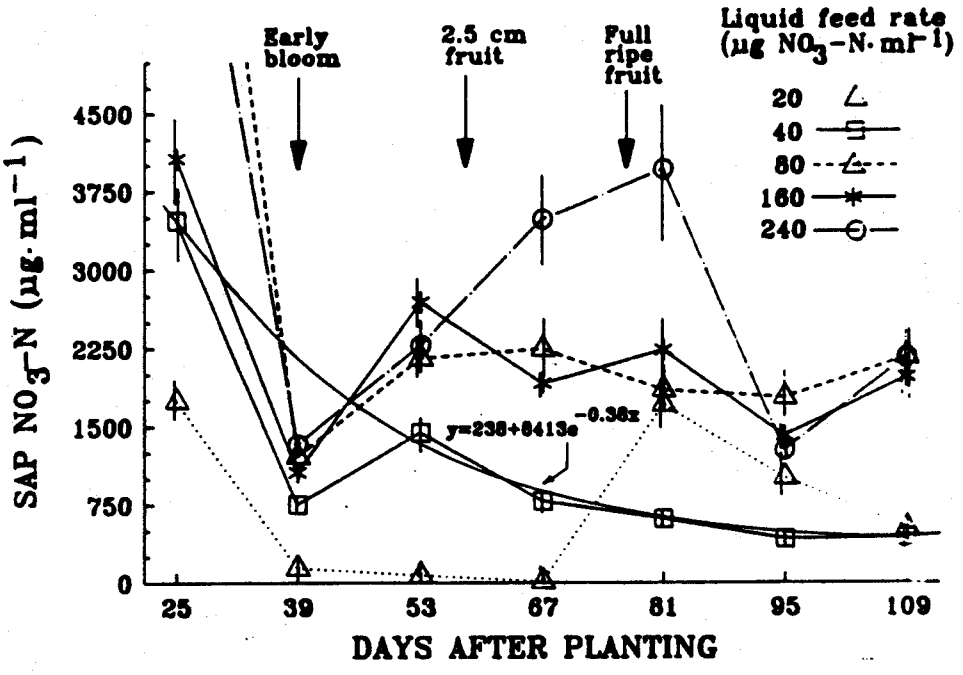

Fig. 15. Sap NO_3-N levels in tomato petioles from plants grown at five N levels (Coltman, 1987a).

A dip in the K content was not found in another experiment of Coltman and Riede (1992; Figure 16).

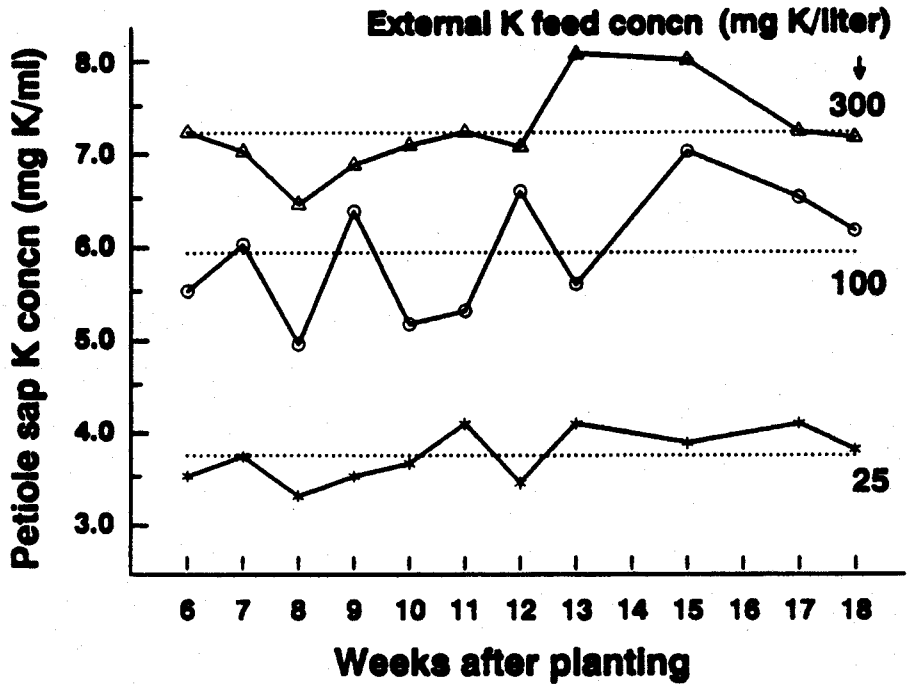

Fig. 16. Petiole sap concentrations over time of tomato plants (Coltman and Riede, 1992).

They concluded that K in petioles of tomato could be used for fertilization schemes. Sampling has to be carried out regularly.

Scheunemann and Paschold (1989) found year to year variation in NO_3 in petioles of white cabbage, late carrot, gherkin and autumn leek for the years 1982-1985. They noted that in sunny summer the concentration was less than in rainy summers. NO_3-content also decreased rapidly from the start of the cropping time until the harvest.

3.4 Other environmental factors

Factors involved are O_2 supply, water supply, N-mineralisation and air humidity.

Low O_2 supply caused a decreasing NO_3 content in petioles of cotton (Constable et al. 1991).

NO_3 in sap of lettuce at high air humidity was somewhat higher than at low air humidity, but the differences were small (Scaife et al., 1985, Annual Report NVRS).

Kroon (1990) and Willemse (1991) suggest a lower NO_3 content in petioles of potato after a long dry period, which was confirmed by results from Verwer et al. (1990).

Long (1982) reported that nitrate levels in sap can be low when there is a lack of soil moisture. In dry period nitrate cannot move to plant roots. He suggested that it is a good idea to stick a cane next to the tested plants, water them and re-test the next day. If they still show nitrate deficiency then it is not caused by lack of water.

Scaife and Turner (1981 Annual Report) did sap measurements on Brussels sprout two days after irrigations. After a first irrigation the sap nitrate concentration had boosted dramatically due to better nitrate uptake in the irrigated plants. After the second, the situation was reversed, the irrigated plants having lower nitrate readings than the non-irrigated possibly due to the extra soil nitrate depletion resulting from the much stronger growth on the irrigated plots at that time.

Scheunemann and Paschold (1989) found on soils rich in organic matter higher NO_3 in petioles than on sandy soils. Probably the N-mineralisation caused higher N uptake in organic rich soils.

3.5 Internal plant factors

A factor mentioned by Coltman (1987a) is the amount of leaves on a tomato plant (Fig 17). In week 13 many leaves were removed and after that time there was a tremendous rise of the NO_3 content in petioles. To investigate this effect of the removal of leaves at another time in week 23 more leaves were removed than normal. Also after this removal rise of the NO_3 content was found. A possible cause for this is not mentioned. It is stated that this effect could cause problems with the interpretation of results.

Another factor to consider is the cultivar. Very little information is available. Martinez et al. (1981; cited in Alcarez et al., 1982) found no differences between cultivars.

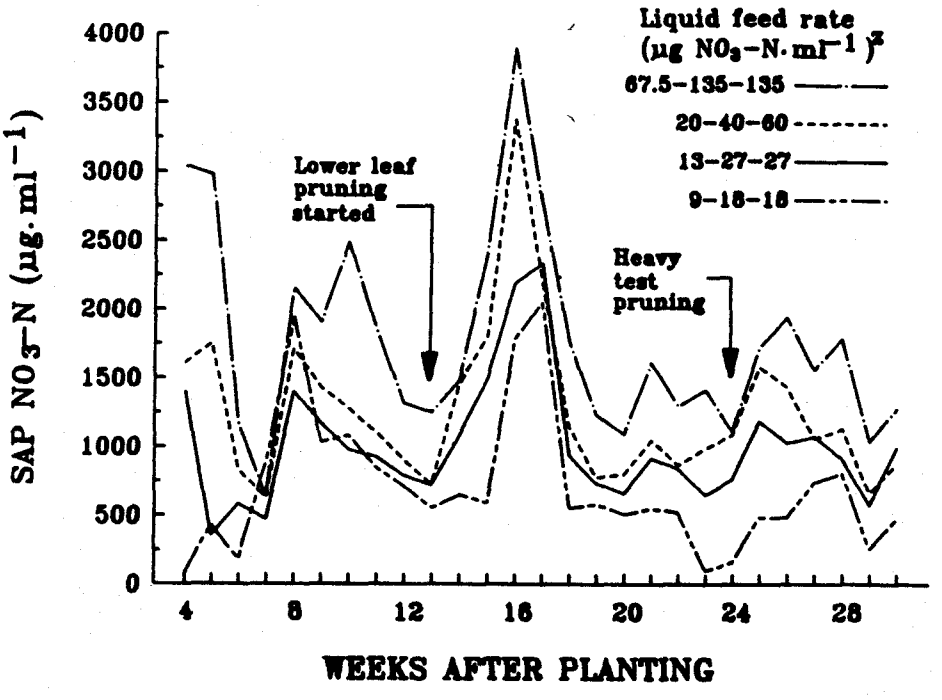

Fig. 17. Sap nitrate in petioles of tomato (Coltman, 1987a).

4. SAP CONTENTS IN ARABLE CROPS AND VEGETABLES IN THE OPEN

A lot of research has been done by the National Vegetable Research Station (NVRS) in the United Kingdom. In summer barley the optimal K concentration was 200 mM (Leigh and Johnston, 1983a). The optimum NO_3-N content in the wheat stem was 904 mg/l at start of the growing period and at harvest it was zero (Scaife, 1979).

For wheat optimum sap content was 30-130 mg/l NO_3-N, 130-280 mg/l P and 1810-4340 mg/l K according to Hernando and Cadahiá (1974).

Routchenko (1971) found the following concentrations in mmol/l for maize NO_3-22; NH_4-4; H_2PO_4-0.8; SO_4-6; Cl-32; K-108; Ca-3; Mg-12; Na-0.3.

Delmas et al. (1959) sampled potato (Bintje) every 2 days from 3 June until 17 June. NO_3-N decreased from 250 to 60 mg/l, P from 40 to 10 mg/l, K increased from 1500 to 2800 and then decreased to 1000 mg/l. Mg was constant at 200 mg/l. Cl was between 2000 and 4500 mg/l. They suggested optimum levels of NO_3-N-225, P-160, K-6000, Mg-400 and Cl <5000 mg/l.

Leigh and Johnston (1983b) found in summer barley always an amount of cations between 250-500 mmol per kg sap. Only a very short time before harvest this increased to 1000 mmol/kg sap. With optimal K fertilization the ratio of these cations were K 70-85%, Ca 15-20%, Mg 3%, Na 1%. If K status of the plant was low the ratios were K 35-40%, Ca 35-40%, Na 25-35%, Mg 5% which was not influenced by N or P fertilization. The osmotic value of the sap was 500 milliosmol per kg sap and in the last 20 days before harvest it increased to 2000 milliosmol per kg sap.

Scaife (1979) stated target values for annual crops (Table 25).

Table 25. Target values for NO_3 in annual crops (Scaife, 1979)

Period	NO_3-N mg/l
first 1/4 growing period	900
second 1/4 ,, ,,	675
third 1/4 ,, ,,	450
last 1/4 ,, ,,	225

Sanchez Conde and Azuara (1980) found in sap of maize the concentrations stated in Table 26.

Table 26. Concentrations in stem of maize (Sanchez Conde and Azuara, 1980).

	stem lowest 2 nodes	rest of plant
NO_3-N, mg/l	1062	587
amino-N, mg/l	343	414
protein-N, mg/l	29	53
NH_4-N, mg/l	74	113
H_2PO_4-P, mg/l	309	361
protein-P, mg/l	26	72
sugars, mg/l	13937	7625
K, mg/l	-	4765
Mg, mg/l	-	316

Willemse (1991) and Kroon (1990) defined optimal NO_3 levels in petioles of potato in relation to days after emergence. The target value 21 days after emergence was 1425-1580 mg/l NO_3-N, 70 days after emergence it was 300-380. The day of emergence was regarded as the day that 80% of the plant emerges.

5. SAP CONTENTS IN VEGETABLES UNDER GLASS

In Table 27 sap contents of different authors are mentioned.

Table 27. Sap contents in tomato; contents in mg/l sap

Source	NO_3-N	NH_4-N	P	K	SO_4-S
A	600-1500	-	200-400	3000-8000	
B	900-1000	<10	120-140	4500-5500	20-40
C	2000-3500	<100	200-300	3000-4500	
D	475		65	4670	
D-	222		44	3740	
E	470		67		
F	900-1500			4000-5500	
G	500				
H	1000-2000				
I	1500-2000		100-150		
J	1000		200		
K	2000-2500		>200		
L	850-1500				
M	>800			5700-6100	
N	225-550	20-150	100-200	3000-4000	
O	1260-2940		280-600	3100-5083	130-550
P	300-500	80	60-90	2600-3000	
Q	2944		15	6770	

Source	Ca	Mg	Cl	Na
A	750-1500	300-1200	<2000	
B	60-100	120-160	-	10-20
C	50-100	100-150		
D			<4200	<1300
N	20-100	100-150		
O	900-2200	700-1700	890-1420	230-800
P	1000	200		200
Q	410			190

A = Lucas and Wittwer (1963) petiole under last truss, after freezing and extraction with 2 % acetic acid.
B = Routchenko (1967) petiole at first truss, one month after flowering.
C = Morard and Kerhoas (1987), side shoots
D = Hernando and Cadahia (1974)
D = Hernando and Cadahia (1974) deficient
E = Values in Spain
F = Drews and Fischer (1990/1991)
G = Maynard et al. (1976); 46 days after sowing
H = Benton Jones et al. (1991) general target value
I = Emmert (1931) before flowering
J = Emmert (1931) at first flowering
K = Emmert (1931) at further flowering

L = Drews and Fischer (1992)
M = Coltman (1987 and 1992)
N = Morard et al. (1983), side shoots
O = Sarro et al. (1985) during week 4-15 after planting
P = Azuara et al. (1982); young plant at flowering of first truss
Q = Hernando and Cadahia (1974), some Cl in the water

Sonneveld (1980) sampled cucumber and tomato on nurseries with rockwool cultivation and cultivation in soil. Petioles of young fully grown leaves had the concentrations shown in Table 28. Cultivation in soil gave higher Na and Cl, both in tomato and cucumber, higher Mg in cucumber, lower NO_3 in cucumber, higher Ca in tomato and lower P, Mn and Zn in tomato than cultivation in rockwool.

Table 28. Macro-elements (in mmol/l) and micro-elements (in mg/l) in petioles of young fully grown leaves (Sonneveld, 1980)

	Cucumber	Tomato
Na	1.0 - 5.1	2.1 - 8.5
K	112 - 138	143 - 176
Ca	19.5 - 20.0	7.0 - 23.6
Mg	3.9 - 6.1	8.9 - 16.7
NH_4	0.7 - 4.3	1.3 - 2.4
NO_3	68 - 127	70 - 111
Cl	3.6 - 64.1	22.1 - 572
SO_4	4.2 - 12.1	8.6 - 19.3
P	4.8 - 8.2	8.7 - 18.1
Fe	0.2 - 1.3	0.3 - 0.8
Mn	0.5 - 1.2	1.0 - 7.8
Zn	0.4 - 1.2	1.4 - 6.0
B	0.2 - 0.4	0.3 - 0.8
Cu	0.1 - 0.8	0.3 - 0.6

Voogt (1982) sampled leaf and petiole of cucumber and eggplant (Table 29). Eggplant had lower Ca content in petiole than in leaf, and cucumber had lower Mg content in petiole than in leaf. Eggplant had lower Ca and higher K content in petioles than cucumber.

Table 29. Leaf and petiole sap contents (Voogt, 1982)

	Sap contents, mmol/l			
	Na	K	Ca	Mg
Cucumber leaf	1.8-2.2	77-106	12.3-19.4	6.4-27.6
petiole	1.2-1.6	95-116	11.1-21.2	2.4- 8.0
Eggplant leaf	0.9-1.6	198-218	9.6-21.1	9.6-22.2
petiole	0.4-0.6	196-212	5.4-11.5	5.4-24.7

Mansson (1978, personel communication) has the same minimum and maximum values for tomato and cucumber (Table 30). Only the target values differ.

Table 30. Target, minimum and maximum values for tomato (Mansson, 1978).

	Base leaf			Top leaf		
	min.	max.	optimal	min.	max.	optimal
NO_3-N, mg/l	600	3200	2300	1000	2200	1700
H_2PO_4-P, mg/l	100	600	400	120	420	300
SO_4-S , mg/l	140	700	400	100	400	250
K	3000	7500	5500	3000	6750	5500
Mg	160	1520	800	160	1000	400
Ca	-	-	1600	-	-	1000
Mn	-	-	5	-	-	3

In the basal leaf concentrations should be higher or equal than in top leaf. For cucumber the values are the same, except that optimal values for base leaf are K - 4500, Mg - 500, Ca - 2200, Mn - 1.5 and for top leaf K - 4500, Mg - 300, Ca - 1200 and Mn - 1.2 mg/l.

Optimal NO_3 content in petioles of young leaves of cucumber is 1020-1245 mg/l NO_3-N (Schacht and Schenk, 1990).

Scheunemann and Paschold (1989) have optimal NO_3 content for white cabbage and late carrot. These values are valuable for sandy soils. In organic soils optimal values are higher.

Table 31. Optimal NO_3-N contents (Scheunemann and Paschold, 1989)

White cabbage

Days from planting	Stage	Optimal NO_3-N mg/l
23 ± 3	8 - leaf	1760 - 1950
48 ± 8	headforming	680 - 790
68 ± 8	head Ø 60 - 100 mm	450 - 565
89 ± 8	head Ø 100 - 150 mm	360 - 475
110 ± 10	head > 150 mm	385 - 520
Late carrot		
40 ± 8	6 - leaf	1360 - 1515
65 ± 10	8 - leaf	815 - 1000
86 ± 10	root < 30 mm	135 - 271
107 ± 10	root > 30 mm	250 - 360

The ranges are very wide for the late carrot. Also for gherkin and autumn leek, data are available.

Drews and Fischer (1991) gave optimal values in petioles of the 5th leaf from the top for tomato and cucumber of 4000-5500 mg/l K and 900-1500 mg/l NO_3-N (tomato) and 1000-1600 mg/l NO_3-N (cucumber). In petioles of cucumber K contents were 1000, 3000, 3500-5000 mg/l for

leaves with deficiency symptoms, leaves without deficiency symptoms but yield depresses and healthy leaves, respectively (Adams, 1982).

Huett and Rose (1988) found optimum NO_3-N of 1000 mg/l in petioles of tomato of youngest fully opened leaf.

Azuara et al. (1982) found in young tomato plants amino-N 200-300, protein-N 100, protein-P 160-260, total-N 900-1000, total-soluble-P 300 and sugars 7000-13000 mg/l.

On Guernsey tomatos are planted in December, first fruit is picked in March and production is year-round. Optimal values (cultivar 'Counter') are given in table 32.

Table 32. Optimal values for petioles of 'base' and 'mid' leaves of tomato. EC is the values measered in the 10 times diluted sap. Other values are in the undiluted sap (Smith, 1987).

	March		April		May		June	
	base	mid	base	mid	base	mid	base	mid
EC, uS/cm	2300	1900	2200	1900	2000	1700	2000	1700
NO_3-N, mg/l	1800	1000	1600	1000	1000	800	1000	700
P	500	400	550	450	550	450	550	450
K	6800	6200	6500	6200	6300	6000	6000	5500
Ca	900	600	900	600	900	600	1000	600
Mg	750	400	750	400	600	400	700	500
Na	200	150	200	150	250	200	300	200
Fe	2.0	1.5	2.0	1.5	2.0	1.5	2.0	1.5
Cu	0.8	0.7	0.9	0.7	0.9	0.9	0.9	0.9
Zn	9.0	5.0	8.0	4.5	8.0	4.5	7.0	4.0
Mn	9.0	7.0	10.0	8.0	12.0	10.0	12.0	10.0
B	4.2	3.5	4.2	3.5	4.2	3.5	4.6	4.2

Values in Table 32 are from crops grown in rockwool. Peat cultivation gives lower P contents, namely 150-250 mg/l P. The Ca:Mg ratio has to be 1.7 : 1.0. The optimum value for Mn is (Ca/200) + (Mg/120). Maximum Na content is 400 mg/l. Fe deficiency appears at Fe < 0.1 mg/l. Zn values lower than 1.5 mg/l caused Zn deficiency. Smith (1987) found Ca levels dependent on age of leaves and plant (Table 33).

Table 33. Optimum Ca levels in petioles (Smith, 1987).

	Optimum Ca, mg/l
Tomato, old leaf	900
Tomato, mid leaf	600
Cucumber old leaf	800 - 1400
Cucumber mid leaf	360 - 1000
Carnation	200 - 500
Gerbera	320 - 760
Rose	320 - 600

Optimal ranges are wide. Azura et al. (1982) desired values of 1000 mg/l Ca in the whole plant.

Prasad and Spiers (1985) defined optimal NO_3 content in tomato petioles, 6 weeks after planting of 900-1100 mg/l NO_3-N, and for 8 weeks after planting of 1100-1400 or 700-800 mg/l NO_3-N. The last values were related to two different trials.

Vielemeyer (1991) found in mid-rib of old still functioning lettuce leaves 226, 900, 1800 mg/l NO_3-N, corresponding with 500, 2000 and 3000 mg NO_3 per kg fresh weight. In the midrib NO_3 was about double as in the whole plant.

Maynard et al. (1976) defined optimum values for NO_3 (table 34).

Table 34. Optimum NO_3 contents. Deficiency appears when concentrations are 10 % lower than optimum (Maynard et al., 1976).

Crop	Date of sampling	Plant Part	NO_3-N, mg/l
Cucumber	42 days after sowing	mature petioles	2000
Lettuce	harvest	whole plant	2000
Radish	harvest	root	500
Spinach	harvest	entire plant	1700
Melon	42 days after sowing	mature petiole	3000

Optimum contents for cucumber are 1000-1600 mg/l NO_3-N and 4000-5500 mg/l K (Drews and Fischer, 1990/1991).

Routchenko (1967) used for cucumber in the 22-leaf stage optimum values of NO_3-N 900-1200; NH_4-N 10-20; H_2PO_4-P 50-100; SO_4-S 20-30; Cl <2500; K 4000-5000; Ca 150-200; Mg 150-250; Na 40-60 mg/l.

Morard and Kerhoas (1987) used for cucumber NO_3-N 2000-3500; NH_4-N <100; H_2PO_4-P 200-300; K 3000-4500; Ca 80-140; Mg 70-120 mg/l as optimum. Morard (1987) had different optimum values for summer and winter planting (Table 35).

Table 35. Optimum values in sap of strawberry (Morard, 1987).

	Winter planting	Summer planting
NO_3-N, mg/l	1000 - 1200	350 - 500
NH_4-N	< 50	< 50
H_2PO_4-P	30 - 38	45 - 75
SO_4-S	16 - 30	16 - 30
Cl	200 - 300	500 - 700
K	4600 - 5600	4600 - 5000
Ca	900 - 1100	750 - 1000
Mg	450 - 600	350 - 500

Burns (1992) gave critical concentration for lettuce (Table 36).

Table 36. Critical concentration in lettuce (Burns, 1992).

	young petiole	middle petiole
NO_3-N, mg/l	95	455
P	106	52
K	1660	2470

Optimum values for lettuce are 120-500 mg/l P and 4000-8000 mg/l K (Burns and Hutsby, 1984).

Ludwich (1990, cited in Benton Jones et al., 1991), mentioned optimum and deficient values (table 37). Optimum values decrease with time of the growing season.

Table 37. Optimum and deficient values (Ludwich, 1990).

Plant	Time	Plant Part	N-content, mg/l Deficient	Optimum
Cucumber	early fruitsetting	petiole of 6th leaf from top	5000	9000
Sweet pepper	early growth	petiole young fully grown leaf	8000	12000
	early fruitset	petiole young fully grown leaf	3000	5000
Tomato	early growth	petiole of 4th leaf from top	8000	12000
(canning)	fruit 1 inch	petiole of 4th leaf from top	6000	10000
	fruit first color	petiole of 4th leaf from top	2000	4000

Lorentz and Bartz (1968) proposed for lettuce at heading optimum values in sap of the midrib of 4000 mg/l NO_3-N and 2000 mg/l P. For tomato at early flowering the optimum values in petiole of 4th leaf from the top are 2000 mg/l NO_3-N and 1500 mg/l P.

Coltman (1987a and b) made the optimum values dependent on the age of the plant of the growing season (Table 38).

Table 38. Optimum values in sap of petiole of young fully grown leaf of tomato (Coltman, 1987 a and b).

Week after planting	Time	Optimal NO_3-N, mg/l
4	flowering	517 - 733
6	fruit 2 - 5 cm	478 - 915
8	fruit 5 - 9 cm	167 - 912

Coltman (1987a and 1987b) introduced different optimum values with different fertilizer management. When fertilization was interrupted NO_3 content of tomato should be higher than with constant fertilization (Table 39).

Table 39. Optimum NO_3 content in petiole of young fully grown leaf of tomato under irregular or constant fertilisation (Coltman, 1987[a], 1987[b]).

Time	Optimal NO_3-N, mg/l	
	Irregular	Constant
Early flowering	2138	517-733
Small fruits (2.5 cm diameter)	1091	478-915
fully ripe fruit	636	167-912

Paschold and Scheunemann (1989) have given the optimal NO_3 content of white cabbage relative to the day after planting (Fig 18).

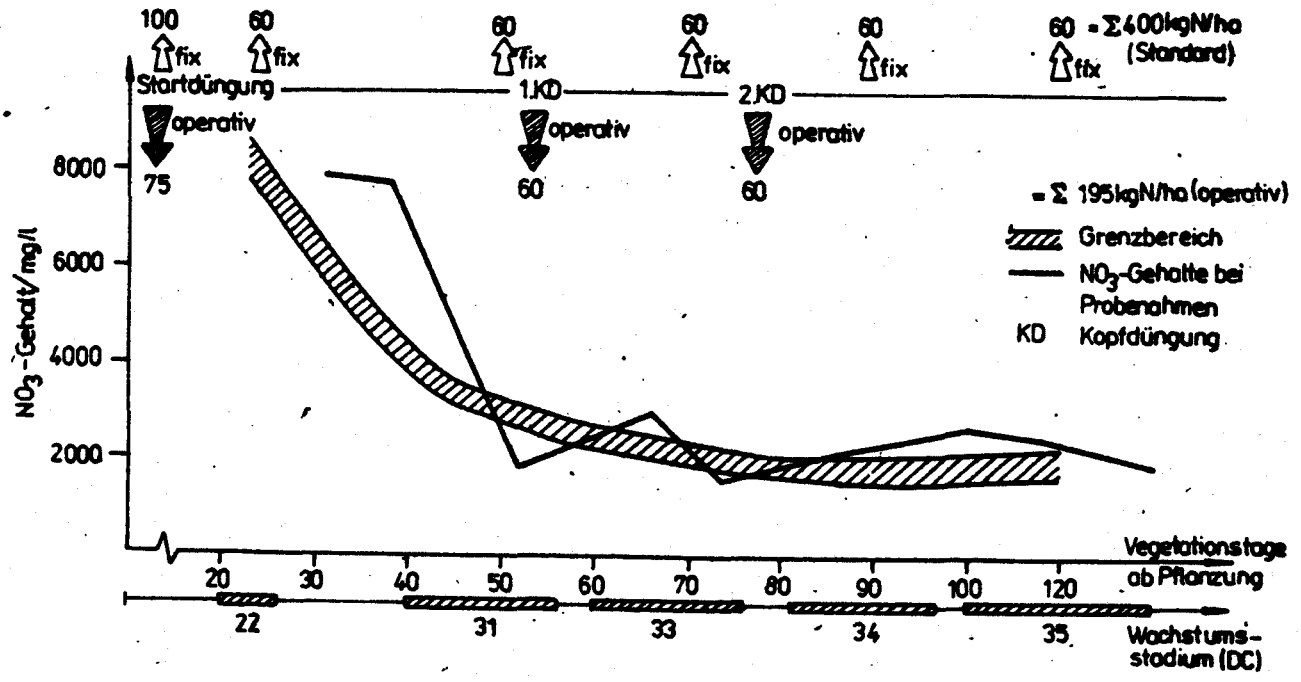

Fig. 18. NO_3 in sap of cabbage (Paschold and Scheunemann, 1989).

Morard et al. (1991) have given optimum values in side shoots of cucumber: Ca 50-200; Mg 50-150; H_2PO_4-P 64-128; NH_4-N 62-155; K 3500-4500, NO_3-N 452-1131 mg/l.

Garcia and Galinier (1989) in France found it better to make optimum NO_3 levels for tomato which was dependent on the daily incoming sun energy. The higher the sun energy the lower the NO_3 content. For example:

y= -2.75 x + 653 where

y= NO_3-N (mg/l) of side shoots of tomato,

x= mean daily incoming radiation of four days just before sampling.

Different values for south west and south east are given in Table 40.

Table 40. Different optimum values in side shoots of tomato for south west and south east in France (Garcia and Galinier, 1989).

Region	NO_3-N	NH_4-N	P	Ca	K	Mg
South west	430-610	35-50	186-265	175-250	3900-5500	150-200
South east	560-705	-	195	260-365	4580-5520	170-200

6. SAP CONTENTS IN CUTFLOWERS AND POT PLANTS.

Prasad and Spiers (1982) defined optimum NO_3 contents in petioles of poinsettia (Table 41), zinnia, davallia, fittonia, Cyclame and geranium. It was very important which criterium was taken for the optimum concentration, it could be growth rate (=increase in height and width of the plant) or increase of dry weight of the plants half way or at the end of the growing period. In the second growing period optimum NO_3 content was always lower than in the first period. Variations were high, for example for zinnia from 560 to > 1600 mg/l NO_3-N and davallia 40-70 mg/l NO_3-N.

Table 41. Optimum NO_3-contents in petioles of poinsettia (Prasad and Spiers, 1982).

	Basis	Optimum NO_3-N mg/l
First growing period	growth rate	260-820
	dry weight	440-1000
Second growing period	growth rate	270-510
	dry weight	250-440

Haarstrich (1988) defined NO_3 content in petiole of Cyclamen of 180-1360 mg/l NO_3-N. The best quality Cyclame was found when fertilization was started at contents in petioles of 2500 - 3500 mg/l NO_3.

For petunia 0 to 1800 mg/l NO_3-N was found (Wollring und Köhler, 1989). For azalea optimum was 300-900 mg/l amino-N and a target value of 450 mg/l amino-N. Mansson used the target values mentioned in Table 42.

Table 42. Target values for poinsettia and rose (Månsson, personel communication).

	Poinsettia	Rose
pH	5.7	5.0
EC, mS/cm	12.5	10.0
NO_3-N, mg/l	195	120
NH_4-N, mg/l	0	0
P, mg/l	680	550
K, mg/l	4400	5700
Mg, mg/l	515	400
S, mg/l	350	350
Ca, mg/l	1100	800
Na, mg/l	34	20
Cl, mg/l	782	400
Mn, mg/l	10.5	7.0
B, mg/l	2.9	4.0
Cu, mg/l	0.8	0.6
Fe, mg/l	3.4	4.0
Zn, mg/l	4.5	5.5
Mo, mg/l	0.89	0.25
Al, mg/l	0.25	0.70

For roses Smith (1987) mentioned optimum values in leaves as follows NO_3-N 50-100; P 200-500; K 3000-4500; Ca 300-600; Mg 250-500; Na 40-100; Fe 3-6; Cu 1-4; Zn 2-5; Mn 5-20; B 5-20 mg/l. In a personnel communication he mentioned some other values: NO_3-N 100-200; Ca 500-800; Mg 60-150; Na 400-600 mg/l.

For geranium optimum NO_3 content was 1000 mg/l NO_3-N (Figure 19; Benton Jones, 1985).

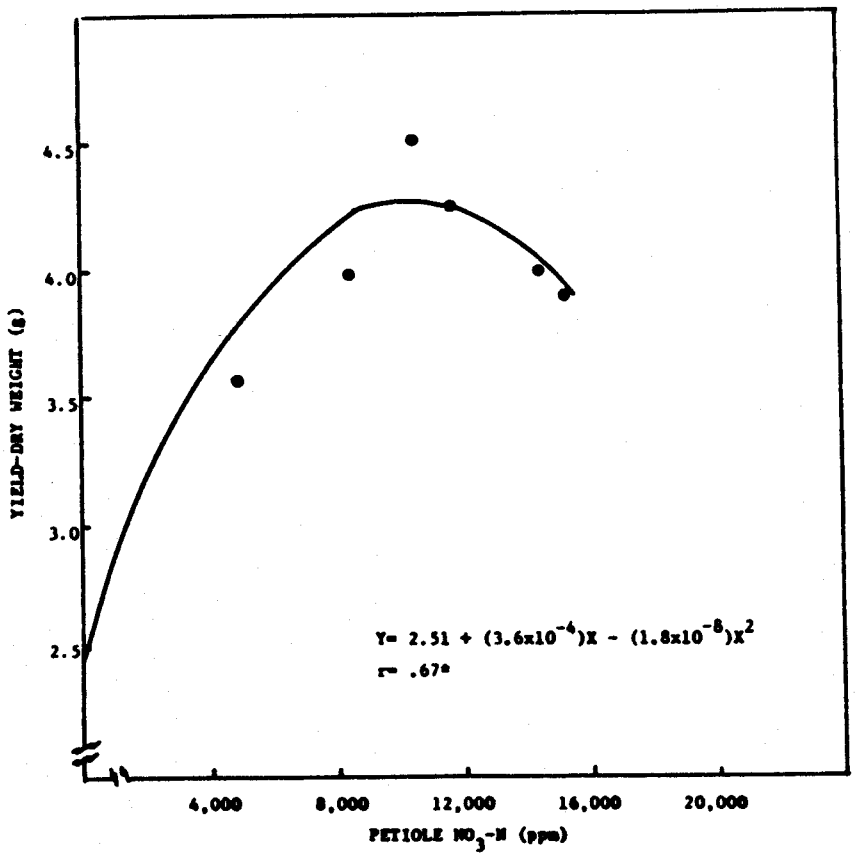

Fig. 19. Relationship between geranium yield and petiole nitrate-N concentration (Woodson and Boodley, 1983; cited by Benton Jones, 1985).

7. COMPARISON 'DRY MATTER TOTAL METHOD' VERSUS 'SAP ANALYSIS'.

Mostly good correlations were found in the literature between contents determined by 'dry matter method' and 'sap analysis', although it is possible that in the case where poor correlations were found the results were not published. This relationship it also dependent on the element.

Good correlations were always found for K, except when K content by 'the dry method' was expressed per unit of dry weight and the dry weight content varied from sample to sample (De Kreij et al., 1992). If K content is expressed per unit of sap also after determination with the 'dry matter method' then a good correlation was found with the sap analysis. Coltman and Riede (1992) compared K in sap and K by total analysis of petioles of tomato and found good correlations ($r = 0.84$; $p < 0.05$). Adams (1982) found for petioles of cucumber even a higher correlation coefficient ($r = 0.99$). He stated that K by total analysis could be converted to K by sap analysis. Burns and Hutsby (1981) found that both K and Na by total analysis or by sap analysis of lettuce reacted the same way after omitting K or Na from the nutrient solution.

Sonneveld and de Bes (1983) compared dry matter method and sap analysis of petioles and laminae of cucumber, tomato and eggplant, and middle and midrib of lettuce. High correlations were found for P, K, Mg and Ca, except for Ca of cucumber laminae, tomato laminae and eggplant laminae. The possible reason for the low correlation is not mentioned. Of the micro elements high correlations were found for Mn, Zn but not for Fe.

Sonneveld and de Bes (1988) found good relationships between K in sap of tomato leaves and petioles and total-K, when it was expressed as sap contents. For Ca also good relationships were found. Total-Ca can be expressed on dry matter. In another trial with carnation Sonneveld and Voogt (1986) found high correlations for Ca (curvilinear relation; Figure 20), K (linear) and Mg (linear). Petiole sap analysis of cucumber, and tomato in a nursery were compared with total analysis of leaves (Sonneveld, 1980). Taking both crops together high correlations were found for Na, K, Ca, NO_3, Cl, SO_4. For Fe there was a poor correlation. For Mg, P, Zn and B good correlations were found only when the two crops were treated seperately. Mg in sap of petiole of tomato was very high, whereas in dry matter of leaves it was comparable was Mg in leaves of cucumber. The same holds for P, Mn, Zn and B.

Prasad et al. (1987) found a high correlation between NO_3 in petioles of kiwi and total-N in petiole + laminae, determined by digesting dry plant material with acitic acid. De Kreij (1989 and 1990) found high correlations between B in sap and by total analysis of Gerbera and carnation. For Gerbera 18-50% of the total-B in the leaves was present in sap; for carnation it was 40-80%.

Vielemeyer and Weissert (1990) found the amount of Ca in the sap of petioles of young tomato leaves relative to the total amount to be 20-25%. For P, K and Mg the figure was 80-100%.

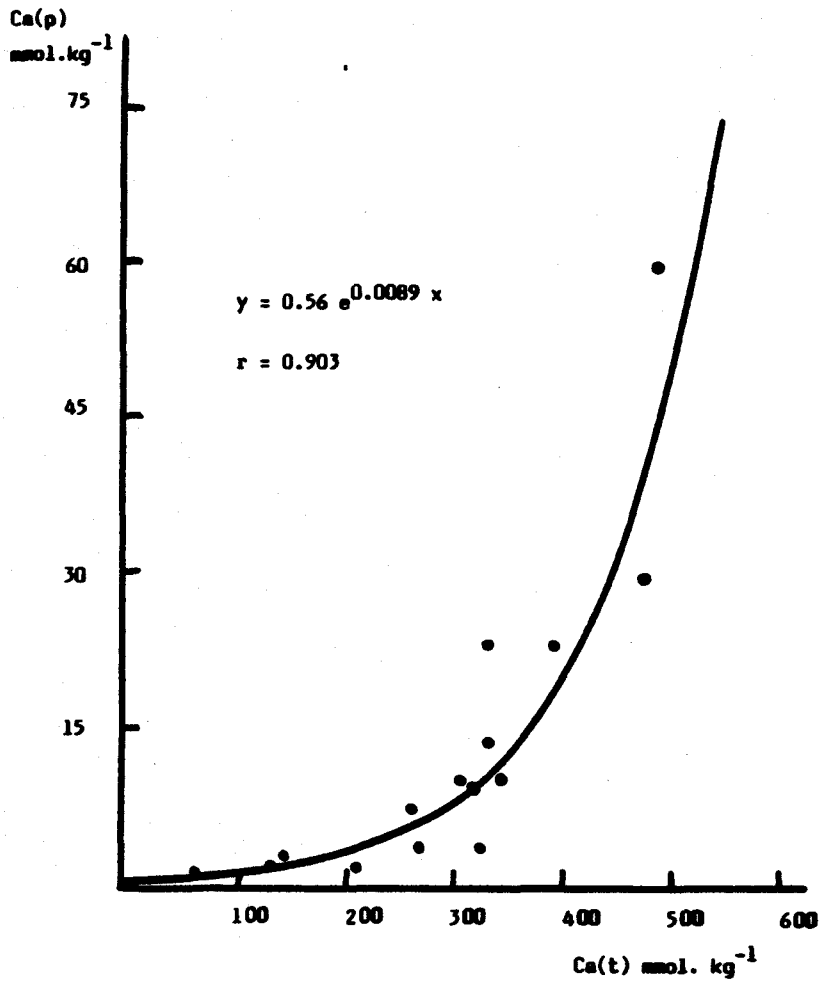

Figure 1 The relationship between the calcium content of carnation leaves determined through digestion of dried material (Ca_t) and that determined through plant-sap analysis (Ca_p).

Fig. 20. The relationship between the calcium content of carnation leaves determined through digestion of dried material (Ca_t) and that determined through plant sap analysis (Ca_p; Sonneveld and Voogt, 1986).

Sometimes plants were analysed by weak acids or other extractants, which gives values somewhere between sap analysis and total analysis values. Extractants used were sodium acetate buffer (Emmert, 1954), 2% acetic acid (Lucas and Wittwer, 1968). Van Lune and van Goor (1979) extracted dried apple fruit with different extractants and gave H_2O-58%; 2M NaCl - 73%; 2M NH_4Cl-77%; 2M HAc - 73%; 2M HCl - 96% of the total Ca.

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